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The Influence of Exogenous Plant Growth Regulators on Sucrose Accumulation in Sugarcane (Saccharum Sp.).

Gerald Matthew Dill Jr
Louisiana State University and Agricultural & Mechanical College

DILL, GERALD MATTHEW, JR.

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THE INFLUENCE OF EXOGENOUS PLANT GROWTH REGULATORS ON SUCROSE ACCUMULATION IN SUGARCANE (*SACCHARUM* SP.)

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Gerald Matthew Dill, Jr.
B.S., Louisiana State University, 1974
M.S., Louisiana State University, 1977
M.Ap.Stat.,Louisiana State University, 1980
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ABSTRACT

The effect of glyphosate on immature internodal tissue of three sugarcane varieties was examined. Sucrose content (mg/g dry wt) increased after treatment application throughout the 7 week period of sampling in the experiment in all varieties examined. Increases in sucrose percent and commercially recoverable sugar per ton (CRS/T) determined by whole stalk analysis were observed in NCo 310 and CP 61-37 4 weeks after glyphosate application. A greater percent increase in sucrose content of immature internodal tissues over control than that observed by whole stalk analysis was noted at this time.

The plant growth regulators glyphosine, ethephon, mefluidide and glyphosate applied to L 62-96 increased the sucrose content of immature internodal tissues examined. Glyphosate appeared to effect a larger increase in this response. The increase observed in sucrose content of these tissues was also observed in sucrose percent and CRS/T at 4 weeks after treatment application. The magnitude of the increase in sucrose content in immature internodal tissues was greater than that observed in whole stalk sugar analysis.

The effect of glyphosate on endogenous glucose and fructose levels in CP 65-357, CP 61-37 and NCo 310 was evaluated. These levels of glucose and fructose declined

throughout the 7 weeks of the experiment. Reducing sugar levels declined more rapidly in glyphosate treated tissues when compared to controls.

Sucrose uptake rates by slices of immature storage parenchyma tissue of sugarcane stalks were examined after glyphosate application. Increased sucrose uptake rates were observed in NCo 310 after glyphosate treatment. This response coincided with increased sucrose content of these same tissues in the experiment. Increases in the rate of sucrose uptake observed in tissue slices of CP 65-357 and CP 61-37 after glyphosate application were not statistically significant.

Neither sucrose percent, average stalk weight nor CRS/T as determined by whole stalk analysis were correlated with seedling in vivo nitrate reductase (NR) activity in the 18 varieties examined. NR activity of greenhouse grown sugarcane seedlings was not indicative of those same seedlings grown in the field. No differences in NR activity were observed between glyphosate treated and untreated commercial varieties throughout the experiment.

PART I. The Effect of Glyphosate on Sucrose Content of Immature Internodal Tissues of Sugarcane.

INTRODUCTION

Plant growth regulators are currently used to increase sucrose content in sugarcane (Saccharum officinarum) at harvest. Screening for such compounds has largely been accomplished via field testing (Nickell, 1977; Martin et al., 1980) using sucrose percent and commercially recoverable sugar per ton of cane determined by whole stalk analysis as evaluating criteria. Fer attempts have been made to determine the effect of plant growth regulators on sucrose content in sugarcane by methods other than whole stalk sugar analysis.

In recent years, glyphosate (N(phosphonomethyl)glycine) has been examined by several researchers for potential commercial use as a plant growth regulator (Clowes, 1980; Martin et al., 1980; McCatty, 1980; Osgood and Teshima, 1980). These authors reported that glyphosate increased sucrose content in sugarcane. The increase in sucrose content caused by glyphosate has been reported greatest in the upper portion of the stalk (Tianco and Gonzales, 1980). By sectioning sugarcane stalks into internode groups (i.e. internode numbers +7 through +15 and +16 to the base of the stalk), these authors observed larger increases in sucrose percent of cane in the

uppermost group of internodes 6 weeks after glyphosate application.

Genotypic differences in response to applied plant growth regulators have been indicated (Alexander, 1976). Varieties have been classified as responding and non-responding with regard to plant growth regulator activity (Martin et al., 1980). The possibility of breeding sugarcane varieties which would be responsive to plant growth regulator application has also been discussed (Martin, 1978).

Since glyphosate causes a larger increase in sucrose content in the upper portion of the sugarcane stalk, this study was undertaken to examine the effects of glyphosate on immature internodal tissues of sugarcane. The objectives of the experiment were to determine: (1) if increases in sucrose content would be observed in these expanding tissues after glyphosate application, (2) whether or not any sucrose response would be consistent with changes observed in sucrose percent and CRS/T as measured by whole stalk analysis and (3) if the response classification of varieties in the experiment, as previously determined by whole stalk analysis (Martin et al., 1980), would be reflected by sucrose levels in immature internodal tissues. It was postulated that analysis of immature internodal tissues could be used as an alternative method of evaluating the response of sugarcane to plant growth regulators such as glyphosate.

MATERIALS AND METHODS

Experimental Design. Experiments were conducted in the fall of 1978 and 1979 at the Agricultural Experiment Station, St. Gabriel, Louisiana on plots of sugarcane (Saccharum officinarum) planted in the fall of 1976 and 1977. The design used in both experiments was a randomized complete block containing 4 replications with a split-split plot arrangement of treatments with sub-plots in strips (Appendix I - Figure 1). Whole plot treatments consisted of single applications of a sodium salt formulation of glyphosate (Appendix I - Table 1) and an untreated control on September 22, 1978 and September 27, 1979 (crop age 5 months). Glyphosate was applied using ground equipment with compressed CO_2 as a propellant in 229 l H_2O/ha to plots 11.1 m x 5.5 m at a rate of 0.45 kg ae/ha. The varieties CP 61-37, NCo 310 and CP 65-357 were planted in strips on the first split while harvest dates (time after treatment application) composed the second split.

The variety CP 65-357 has been classified as responsive to plant growth regulator application (Martin et al., 1980). This variety is also considered early maturing, reaching 12% sucrose prior to the third week in October (Richard et al., 1978). Discussions on the maturity of

sugarcane varieties grown in Louisiana generally refer to commercial harvesting of the crop rather than the physiological state of the plant. The value of 12% sucrose is generally accepted in Louisiana as that point at which harvest is economically feasible (Legendre and Martin, 1976). Sugarcane grown in Louisiana does not reach physiological maturity due to the short growing season (Legendre, 1975). The varieties NCo 310 and CP 61-37 are classified as late maturing varieties generally not reaching 12% sucrose until beyond the third week of November (Richard et al., 1978). NCo 310 has been classified as non-responding with regard to plant growth regulator application while CP 61-37 has not been characterized by this method of classification (Martin et al., 1980).

Analysis of Immature Internodal Tissue. Plots were sampled over a 7 week period after treatment application. Sampling was conducted weekly through the first 3 weeks after treatment application and every other week thereafter. Samples of 3 stalks were cut flush with the ground and transported back to the laboratory. The immature internode +3 was identified as previously described (Van Dillewijn, 1952) and segmented from each stalk. This internode was chosen since elongation was not complete but was large enough that a sufficient amount of storage parenchyma tissue could be obtained from the sample. The

rind was peeled from each internode leaving only immature storage parenchyma tissue, which was quartered lengthwise and cut into 2-3 mm thick sections.

A 20 g subsample of internodal tissue was weighed and placed in 25 ml 95% ethanol and stored at -15 C for 4 weeks (Bieleski, 1960a). The tissue was extracted in 75 ml hot (80 C) 95% ethanol (Bowen, 1972) using a Virtis blender for 3 min. The extraction mixture was poured into a Buchner funnel containing a 5 cm Whatman No. 1 filter. The fibrous residue was then rinsed with hot 95% ethanol (4 x 25 ml), dried and weighed. The liquid fraction containing the stored sugars was transferred to a 250 ml volumetric flask and brought to volume after cooling with distilled H_2O. It was necessary to remove the ethanol from the sample because at such high concentrations this solvent masked the detection of sucrose and reducing sugars in subsequent analysis. A 3.0 ml aliquot was flash evaporated in a Buchler rotary evaporator (Model 3-2100). The sample was brought back to a 3.0 ml volume with an aqeous solution of 0.03M sodium azide to prevent bacterial contamination. This fraction was analyzed for sucrose and reducing sugar content with a liquid chromatography (LC) system developed specifically for sugarcane saccharide analysis (Wong-Chong and Martin, 1979a; 1979b). A Waters LC system with a 7mm ID x 61cm Aminex Q150S (K^+) ion exchange column was used. The system included a Model

M-45 solvent delivery system, Model 710A Wisp automatic sampler, Model R401 differential refractometer, and Model 730 Data Module all manufactured by Waters Associates. A 40 l sample was injected into an H_2O solvent with a flow rate of 2.5 ml/min. Sucrose concentrations were determined by peak area estimation using the Data Module and reported as mg of sucrose per gram dry weight of tissue.

Analysis of Whole Stalk Samples. Data were collected 4 weeks after treatment application. Sufficient time should have elapsed to detect increases in sucrose percent and commercially recoverable sugar per ton of cane caused by glyphosate application (Martin et al., 1980). Samples containing 10 stalks were harvested. Stalks were cut flush with the ground, topped through the vegetative apex, weighed and milled once through a 3 roller mill. For each juice sample, brix (percent soluble solids) was determined using a Sargent Model S-42440-B spindle hydrometer and apparent sucrose with a Rudolf Model IIS Autopol automatic saccharimeter (Meade, 1963). Yield estimates of commercially recoverable sugar per metric ton of cane (CRS/T) were calculated from these data (Dill et al., 1978).

Statistical Analysis. An analysis of variance was run utilizing data collected in both the 1978 and 1979 experiments for the variable sucrose content (Appendix I -

Table 3). Several models were tested to define the response of sucrose content with time for the treatments in these experiments. The effects of a treatment within a given variety (treatment x variety interaction) and the effect of time on the specific treatment and variety combination in question were used in establishing these models. The technique used to construct the tests of hypothesis desired involved the estimation of area beneath glyphosate treated and untreated response curves for each variety examined (Appendix II). Treatment differences were declared significant when the difference in area beneath response curves being compared was declared significantly different from zero using an F ratio. Corresponding single degree of freedom comparisons were made for sucrose percent and CRS/T in these experiments. Overall analysis of variance tables for sucrose percent and CRS/T are shown in Appendix I (Tables 6 and 9). An α level of 0.05 was selected as the criteria for declaring significant treatment differences.

RESULTS

Figures 1-3 show sucrose content of glyphosate treated and untreated CP 65-357, CP 61-37, and NCo 310 respectively. Data shown are averaged across both the 1978 and 1979 experiments. Immature internodal tissues of CP 65-357 showed increased sucrose content after glyphosate application (Figure 1). Maximum response occurred in the latter stages of the experiments. Five weeks after glyphosate application extractable sucrose was highest in these tissues at 474 mg sucrose per gram dry weight of tissue (Figure 1), averaging 74% over control at this time. The increase in the sucrose content of glyphosate treated CP 65-357 was declared significant (Table 1). An increase in sucrose content of immature internodal tissues was also observed for CP 61-37 after glyphosate application (Figure 2). Response curves indicate that an increase over control in sucrose content of approximately 50% occurred between 4 and 5 weeks after glyphosate application. This response was declared statistically significant (Table 1). Similarly, increased sucrose content was observed in NCo 310 after glyphosate application (Figure 3). Increases over control were observed highest in the latter stages of the experiments with a 63% increase in sucrose content of glyphosate treated tissues at 7 weeks after application

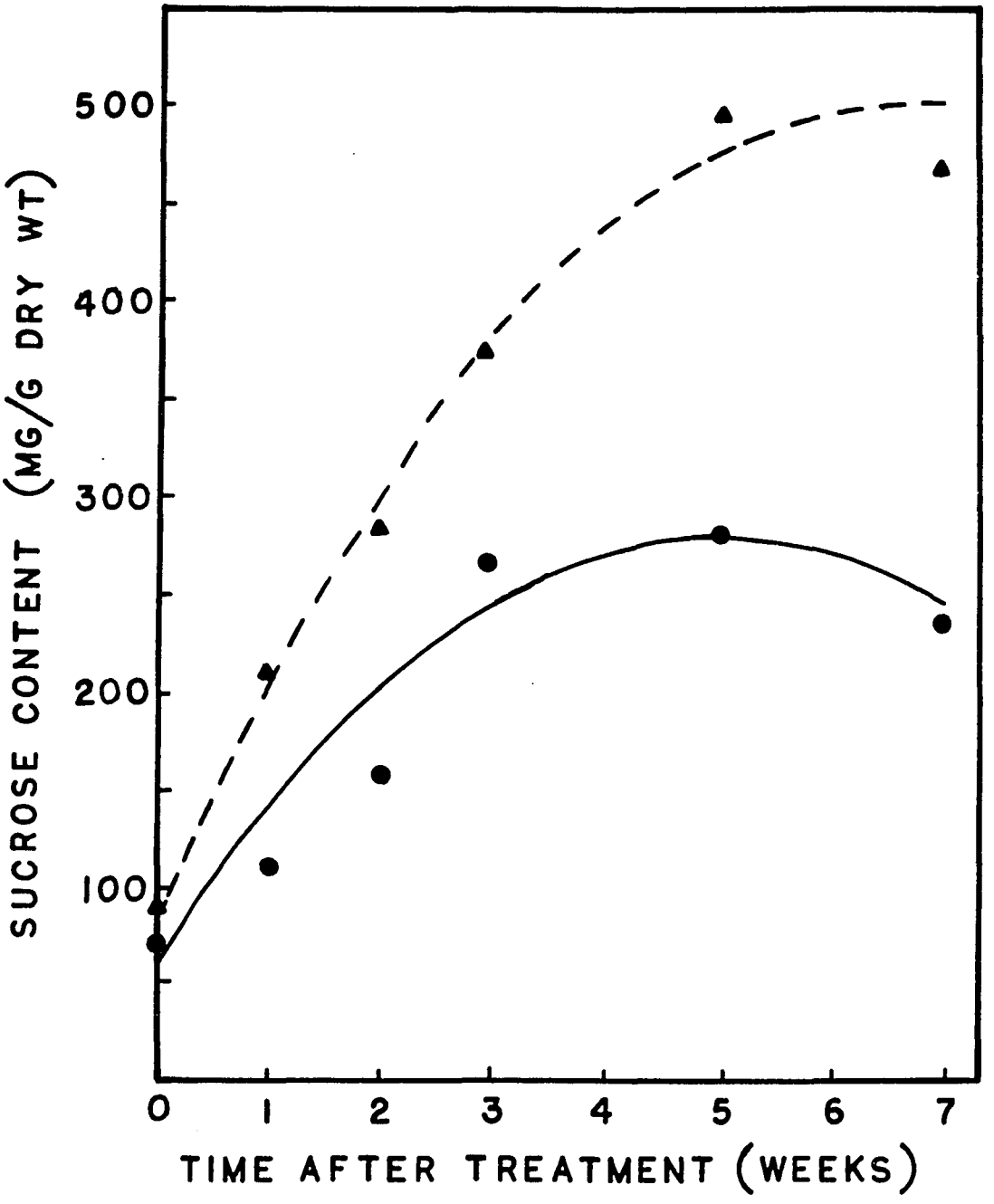

Figure 1. Average sucrose content of immature internodal tissue of glyphosate treated (▲) and untreated (●) CP 65-357 after treatment application.

Table 1. Estimated quadratic response equations generated from sucrose content data collected in the 1978 and 1979 experiments, x = weeks after treatment application.

Variety	Treatment	
	Control	Glyphosate
CP 65-357	$45.36 + 90.32x - 8.90x^2$	$85.56 + 125.14x - 9.49x^2$ *
CP 61-37	$83.82 + 34.87x + 1.35x^2$	$98.88 + 139.51x - 14.50x^2$ *
NCo 310	$48.71 + 63.65x - 4.32x^2$	$68.85 + 122.08x - 9.43x^2$ *

* Significantly different from control at $\alpha = 0.05$ level.

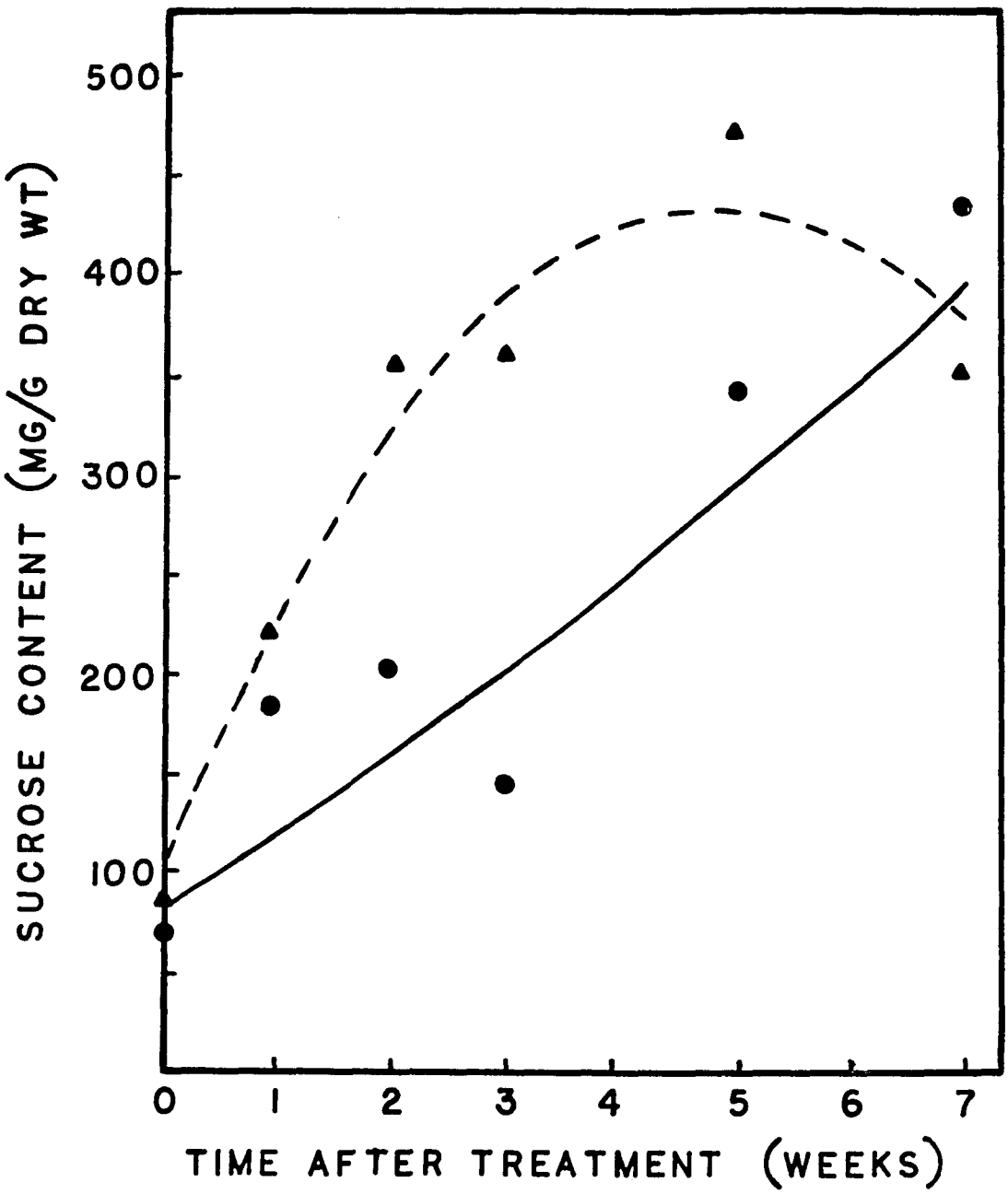

Figure 2. Average sucrose content of immature internodal tissue of glyphosate treated (▲) and untreated (●) CP 61-37 after treatment application.

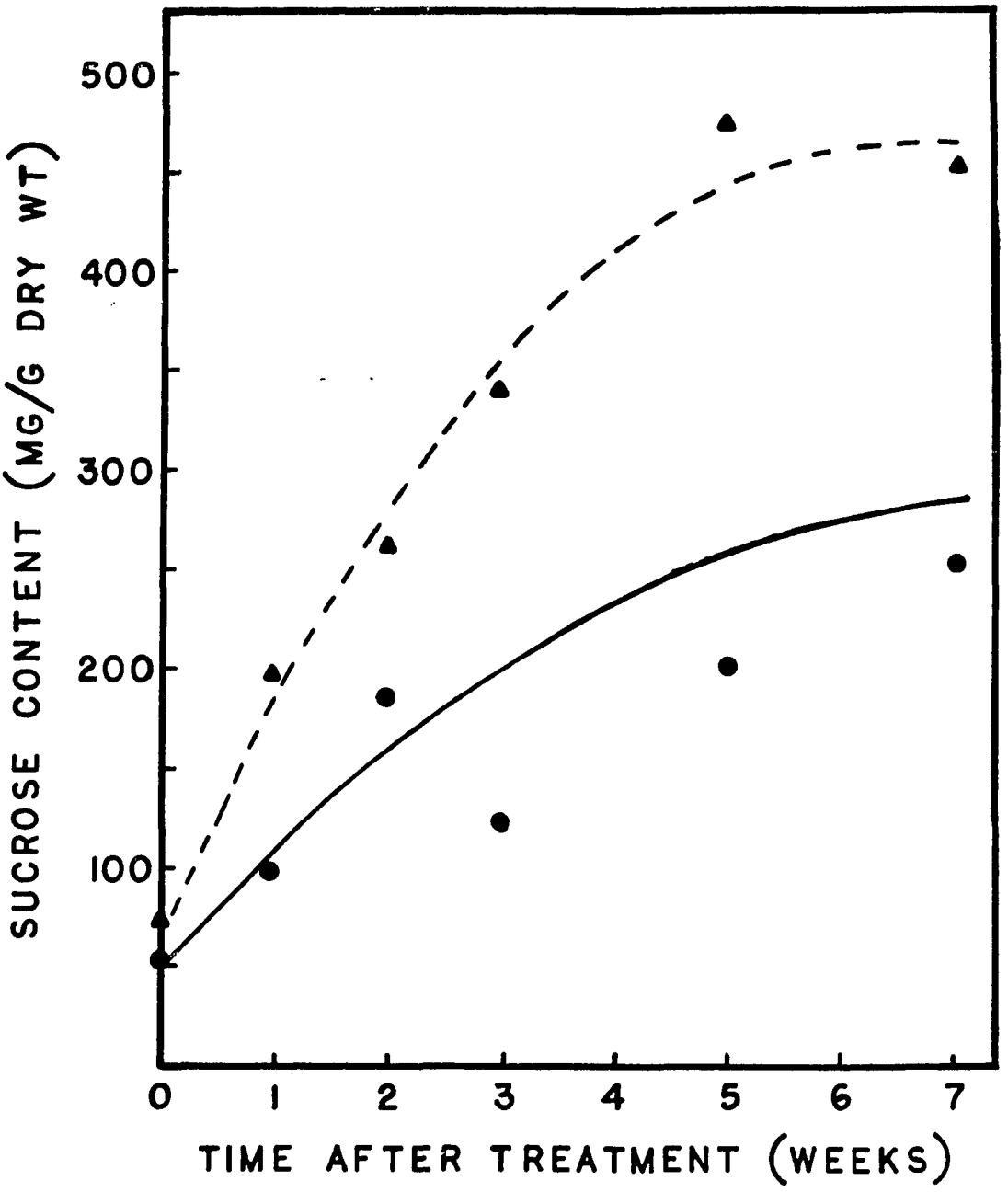

Figure 3. Average sucrose content of immature internodal tissue of glyphosate treated (▲) and untreated (●) NCo 310 after treatment application.

(Figure 3). The observed increase in sucrose content was statistically significant (Table 1). An analysis of variance for sucrose content is shown in Appendix I (Table 3). Means observed in the 1978 and 1979 experiments are also shown in Appendix I (Tables 4 and 5).

Estimates of sucrose percent and CRS/T obtained at 4 weeks after treatment application are shown in Table 2. Also presented are sucrose content (mg/g dry wt) values for immature internodal tissue at this time predicted from the quadratic response equations (Table 1). Increases were observed in sucrose percent of cane and CRS/T for the varieties CP 61-37 and NCo 310 by whole stalk sucrose analysis (Table 2). A 14.3% increase in sucrose percent and a 17.4% increase in CRS/T were observed in the variety CP 61-37 4 weeks after glyphosate application. The predicted sucrose content of immature internodal tissue was much higher than control in this variety (Table 2). Increases in sucrose percent (18.0%) and CRS/T (24.7%) were also observed in the variety NCo 310 along with an increase in the sucrose content of internode +3 after glyphosate application. The increases in sucrose percent and CRS/T were declared significant for both varieties by analysis of variance (Table 2). Increases in average sucrose percent, CRS/T and the sucrose content of immature internodal tissues were observed for the variety CP 65-357. However, differences in sucrose percent and CRS/T were not

Table 2. Sucrose percent and CRS/T observed 4 weeks after glyphosate application in whole stalk analysis averaged across the 1978 and 1979 experiments. Sucrose content (mg/g dry weight) of immature internodal tissues was estimated from quadratic response equations at 4 weeks after glyphosate application.

	Variety								
	CP 65-357			CP 61-37			NCo 310		
Variable	C[1/]	G[2/]	% Diff	C	G	% Diff	C	G	% Diff
Sucrose Content (mg/g dry wt)	264.24	434.28	64.3%	244.90	424.90	73.5	234.19	406.29	73.5
Sucrose Percent (%)	15.00	16.34	8.9	12.90	14.74*	14.3	10.68	12.60*	18.0
CRS/T (kg/ton)	66.61	74.15	11.3	56.02	65.79*	17.4	42.73	53.29*	24.7

* Significant at the $\alpha = 0.05$ level.

1/ C = Control.

2/ G = Glyphosate.

declared significant for this variety (Table 2). Analysis of variance tables are presented for sucrose percent (Appendix I, Table 6) and CRS/T (Appendix I, Table 9) along with means observed in 1978 and 1979 experiments for sucrose percent (Appendix I - Tables 7 and 8) and CRS/T (Appendix I - Tables 10 and 11).

DISCUSSION

An increase in sucrose content was observed after glyphosate application in internodal tissues of all varieties examined in this experiment. The magnitude of this response appeared to be similar in the varieties CP 65-357 and NCo 310 (Figures 1 and 3 respectively). The variety CP 65-357 has been classified a responding variety and NCo 310 a non-responding variety (Martin et al., 1980). While the response of CP 65-357 to glyphosate application observed in this experiment (Figure 1) might be expected, the same increases observed in NCo 310 (Figure 3) are inconsistent with previously mentioned response classification. However, reports have appeared in the literature that NCo 310 can respond to glyphosate application (Clowes, 1980). The response of the variety NCo 310 to exogenous glyphosate application would seem to indicate that criteria used in evaluating the response level of this genotype should be reviewed. Perhaps the susceptibility of NCo 310 to sugarcane mosaic virus (Steib, 1974) could explain this divergence of opinion. Plant growth regulator efficacy has been reported to be adversely affected by ratoon stunting disease (Martin et al., 1980). If such an effect were also true for sugarcane mosaic virus, then the lack of response of NCo 310 to plant growth regulators as previously

reported (Martin et al., 1980) could have been the result of a plant growth regulator and disease interaction. In this experiment care was taken to ensure that seed cane used in planting experiments was disease free.

Sucrose content in immature internodal tissue of CP 61-37 was higher after glyphosate application (Figure 2). Maximum response was observed 3 to 5 weeks after glyphosate application. A decrease in sucrose content of glyphosate treated tissues at the final sampling date resulted in a negative response to glyphosate at that time. This effect is probably the result of tissue damage from dessication rather than an actual decline in the sucrose content of the immature internodal tissues. It should also be noted that a decline in sucrose content after glyphosate application was observed only in CP 61-37.

Estimates obtained at 4 weeks after treatment in whole stalk analysis showed an increase in the average sucrose percent of cane and CRS/T in all varieties tested (Table 2). The predicted sucrose content of immature internodal tissues at 4 weeks after glyphosate application was also higher in all varieties examined. The magnitude of the response observed in sucrose percent ranged from 8.9% in CP 65-357 to 18.0% in NCo 310 (Table 2). Similarly, increases from 11.3% to 24.7% were observed for these two varieties in CRS/T. However, the increase in sucrose predicted from extraction of immature internodal

tissues was much higher, ranging from 64.3% to 73.5% in the varieties CP 65-357 and NCo 310 respectively (Table 2). This may be due to a greater response to glyphosate in young expanding internodes of the upper portion of the sugarcane stalk as has been suggested (Tianco and Gonzales, 1980; Martin et al., 1980). It should also be noted that the increase in sucrose content due to glyphosate in immature internodal tissues (Figures 1-3) are consistent with sucrose percent and CRS/T responses reported from experiments utilizing whole stalk sugar analysis (Osgood, 1979; Martin et al., 1980). This indicates that increased sucrose content in immature internodal tissues in response to glyphosate reflects increases in sucrose percent and CRS/T as measured by whole stalk analysis in CP 61-37 and NCo 310.

Sucrose content in immature internodal tissue was significantly higher than control in CP 65-357 after glyphosate treatment (Table 1). However, no significant increase in sucrose percent or CRS/T over control was noted after glyphosate application in this variety (Table 2). This suggests that increases in sucrose content of immature internodal tissues after glyphosate application do indicate response to treatment, but are not always indicative of results obtained in whole stalk analysis. It should be noted that significant increases over control in sucrose percent and CRS/T were observed in NCo 310 and

CP 61-37 which are classified as late maturing varieties (Richard et al., 1978). These data would suggest a relationship between response to glyphosate and the maturity pattern of sugarcane varieties. However, further experiments involving a large number of early and late maturing varieties would be necessary to determine if late maturing varieties respond more consistently to glyphosate treatment than early maturing varieties.

Increased sucrose content in sugarcane is probably a secondary response to glyphosate. The proposed mode of action of this compound is the inhibition of aromatic amino acid synthesis (Jaworski, 1972; Amrhein, et al., 1980). The rapid translocation of glyphosate to the apical meristem (Takahashi, 1976) and the reduction in the vegetative growth rate of sugarcane after its application (Dill et al., 1980) might indicate a secondary action in altering source-sink relationships within the stalk. This would be consistent with reported observations that reduced turnover rates of existing sucrose in glyphosate treated tissues were probably due to a decline in demand for sugars for processes associated with vegetative growth (Hilton et al., 1980).

The efficacy of glyphosate as a plant growth regulant in sugarcane observed in this experiment was consistent with previous observations (Clowes, 1980; Mason, 1980; Hilton et al., 1980). The increase in sucrose content in

immature internodal tissues in response to glyphosate was larger than that observed in more traditional whole stalk sugar yield estimation. This suggests that glyphosate effects were more pronounced in the upper portion of the sugarcane stalk. The results obtained indicated that sucrose content of immature internodal tissues is indicative of changes in sucrose percent and CRS/T as measured by whole stalk analysis of two varieties in this experiment.

PART II. Sucrose Content of Immature Internodal Tissues of Sugarcane as Affected by the Exogenous Application of Plant Growth Regulators.

INTRODUCTION

Glyphosate (N,(phosphonomethyl)glycine), glyphosine (N,N-bis(phosphonomethyl)glycine), ethephon (2 chloroethylphosphonic acid) and mefluidide (N-(2,4-dimethyl-5-(((triflouromethyl)-sulfonyl)amino)phenyl)acetamide) have been shown to increase sucrose percent in sugarcane (Rostron, 1975; Teshima and Osgood, 1977). Glyphosine was the first plant growth regulator used commercially in sugarcane for sucrose enhancement in the United States. As might be expected, its yield enhancing capabilities have been extensively examined (Martin and Legendre, 1976; Fernandez et al., 1976; Julien et al., 1980). Like glyphosate, glyphosine is classified a growth repressant due to a reduction in the growth rate of sugarcane seen after its application. (Martin et al., 1978). Studies indicating reduced net photosynthetic rates and increased respiration rates of leaf tissue (Dill and Martin, 1977) have also appeared in the literature.

Increases in sucrose percent and accompanying reductions in vegetative growth as measured by whole stalk analysis have been reported after mefluidide and ethephon were applied to sugarcane (Maretzki and Dela Cruz, 1979; Takahashi, 1976). Growth stimulation of sugarcane after ethephon application has also been

reported (Rostron, 1973; Clowes, 1978). Sugarcane varieties 9-11 months old, which were actively growing under conditions described as poor for natural sucrose enhancement, had increased stalk mass after ethephon application (Clowes, 1978). However, the author also reported significant reductions in the sucrose content of whole stalk samples of sugarcane in which this response was observed.

Glyphosate increased sucrose content in immature internodal tissues of the varieties examined in Part I. The first objective of this experiment was to determine if this same effect would be observed for other plant growth regulators. Secondly, it was of interest to compare sucrose enhancement in immature internodal tissues caused by compounds in this experiment and responses observed in sucrose percent and CRS/T measured by whole stalk analysis. It was postulated that examining sucrose levels in the immature internodal tissues of sugarcane could be used in evaluating plant growth regulator response in sugarcane.

MATERIALS AND METHODS

Experimental Design. The experimental design, method of treatment application and sampling are described in Part I. The sugarcane variety L 62-96 was chosen for examination of the effects of glyphosate, glyphosine, ethephon and mefluidide on sucrose content of immature internodal tissues and sucrose percent and CRS/T as measured in whole stalk analysis. This variety has been classified as responsive to plant growth regulators (Martin et al., 1980) and has been described as early maturing (Richard, et al., 1978). Treatments were applied to plots at rates previously described (Teshima and Osgood, 1977). Treatment rates and formulation descriptions are listed in Appendix Table 1.

Statistical Technique. The analysis utilized was essentially the same as in Part I; however, a set of orthogonal comparisons was constructed to examine treatment differences. Four single degree of freedom tests of hypothesis were constructed to compare (1) control vs. the average response to all other treatments in the experiment, (2) glyphosate vs. the average of glyphosine, mefluidide and ethephon responses, (3) glyphosine response compared to the average mefluidide and ethephon responses and,

(4) a comparison of mefluidide and ethephon effects. These comparisons were constructed to compare differences in response among treatments in this experiment. The area estimation technique (Appendix II) used in comparing sucrose content of immature internodal tissues was conducted within the framework of these orthogonal comparisons. An α level of 0.05 was selected for declaring treatment differences significant.

RESULTS

The sucrose content of immature internodal tissues of L 62-96 are shown in Figure 4. Quadratic response equations were estimated for control and plant growth regulator treated internodal tissues from data collected in both 1978 and 1979 (Appendix I - Table 12). All treatments apparently increased the sucrose content during the course of the experiment (Figure 4). Means observed in the 1978 and 1979 experiments associated with these responses are shown in Appendix I (Tables 4 and 5). The analysis of variance for these data is shown in Appendix I (Table 3).

The average response of all treatments compared with control indicated that the plant growth regulators tested effected a significant increase in sucrose content (Comparison 1, Table 3). Glyphosate caused the largest increase in sucrose content in immature internodal tissue on the final two sampling dates (Figure 4). Glyphosate was significantly higher than the average increase in sucrose content of all other plant growth regulators in the experiment (Comparison 2, Table 3). Ethephon effected a larger increase in sucrose content than glyphosine (Figure 4). The increase in the sucrose content with time caused by both of these treatments appeared to level

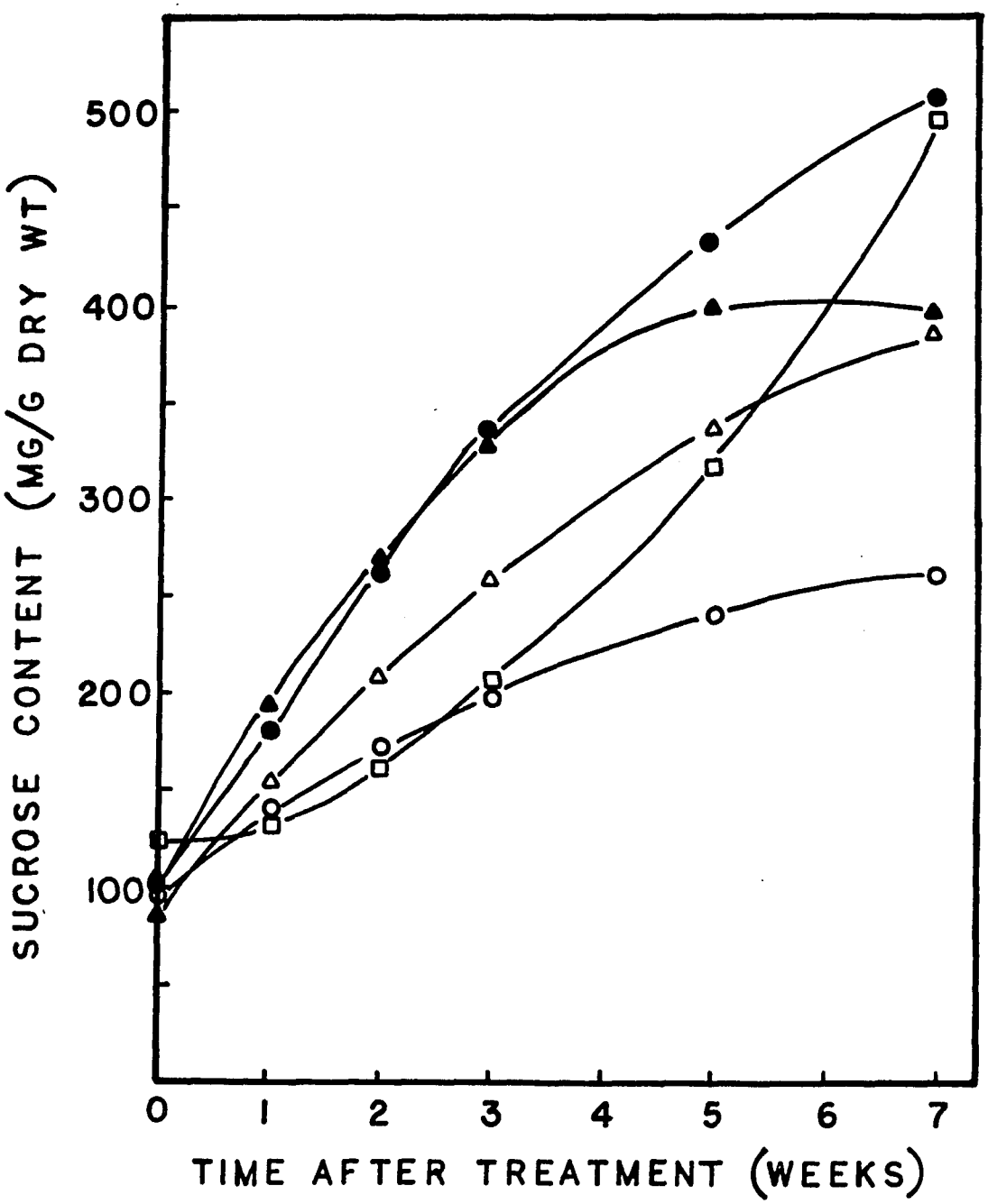

Figure 4. Average sucrose content of immature internodal tissue of glyphosate (●), glyphosine (△), ethephon (▲), and mefluidide (□) treated and untreated (○) L62-96 after treatment application.

Table 3. F values obtained from orthogonal comparisons established for analyzing treatment differences.

Comparison	Sucrose Content[1/] (mg/g dry wt)	Sucrose[2/] Percent	CRS/T (kg/ton)
1. Control vs. All Treatments	28.67*	0.68	0.54
2. Glyphosate vs. Glyphosine, Mefluidide, and Ethephon	10.01*	0.41	0.41
3. Glyphosine vs. Mefluidide and Ethephon	3.78	0.02	0.07
4. Mefluidide vs. Ethephon	4.96*	0.94	1.01

[1/] Comparisons made across all sampling dates for immature internodal tissue.

[2/] Comparisons made at 4 weeks after treatment application (whole stalk analysis).

* Significant at the $\alpha = 0.05$ level.

off at the fifth and seventh weeks after treatment application. Mefluidide caused a substantial increase in sucrose content in the latter stages of the experiment (Figure 4). Average effects of mefluidide and ethephon on sucrose content were no different from glyphosine (Comparison 3, Table 3). A significant difference in sucrose content was observed between mefluidide and ethephon by area comparison (Comparison 4, Table 3). This indicated that ethephon caused a higher increase in sucrose content of immature internodal tissues than mefluidide through the fifth week after treatment application.

Average sucrose percent and CRS/T observed by whole stalk analysis 4 weeks after treatment application are shown in Table 4. Highest sucrose percent and CRS/T were observed in the mefluidide and ethephon treatments representing increases of approximately 8% in sucrose percent and 10% in CRS/T over control. Glyphosate increased sucrose percent by 6.5% and CRS/T by 8% over control. Glyphosine caused the smallest increase in sucrose percent (3.1%) and CRS/T (4.7%) of the plant growth regulators tested. None of the differences in sucrose percent and CRS/T among treatments were statistically significant (Table 3). Sucrose content (mg/gm dry wt) of immature internodal tissue is presented as predicted from the quadratic response equations (Appendix I - Table 12) for

Table 4. Average sucrose percent and CRS/T obtained 4 weeks after treatment application for L 62-96 by whole stalk analysis. Sucrose content of immature internodal tissue was obtained using quadratic response equations at 4 weeks after treatment application.

Treatment	Sucrose Content (mg/g dry wy)	% Difference	Sucrose Percent	% Difference	CRS/T (kg/ton)	% Difference
Control	216.52		13.70		72.76	
Glyphosate	380.42	75.7	14.60	6.5	78.74	8.0
Glyphosine	297.16	37.2	14.12	3.1	76.19	4.7
Mefluidide	249.96	15.4	14.80	8.0	79.95	9.9
Ethephon	368.65	70.3	14.77	7.8	80.12	10.1

4 weeks after treatment application. Highest sucrose content was observed in the glyphosate treatment, representing a 75.7% increase over control (Table 4). Ethephon effected a 70.3% increase while glyphosine and mefluidide increased sucrose content 37.2% and 15.4% respectively over control.

DISCUSSION

The quadratic responses generated for the effect of growth regulators on the change in sucrose content of immature internodal tissue (Figure 4) was consistent with whole stalk sucrose percent and CRS/T responses previously reported (Osgood, 1979; Martin et al., 1980). The increase in sucrose content in tissues of L 62-96 caused by glyphosate application was similar to those observed in the varieties CP 65-357 and NCo 310 (Figures 1 and 3 - Part I).

All treatments appeared to increase sucrose content over control in immature internodal tissues of the variety L 62-96 (Figure 4). This genotype has been reported to be responsive to exogenous plant growth regulator application (Martin et al., 1980). The data observed here supports the classification of this variety as responsive to plant growth regulators. Glyphosate increased endogenous sucrose levels more than other compounds tested particularly in the later stages of the experiment (Figure 4). This would be consistent with earlier work (Teshima and Osgood, 1977). Increases in sucrose content were similar in glyphosine and ethephon treated tissues although sucrose content was higher after ethephon application through the fifth week after application (Figure 4). The response of mefluidide treated immature internodal tissues occurred in the latter stages

of the experiment, through the fifth and seventh week after treatment application (Figure 4). The variety L 62-96 responded differently to the treatments applied in this experiment, not only in the magnitude of response but the time at which increases in sucrose content were observed.

Average sucrose percent and CRS/T observed in whole stalk analysis 4 weeks after treatment application indicated only slight responses to plant growth regulator application (Table 4). The mefluidide and ethephon effected a larger percent change in sucrose percent and CRS/T than did glyphosine and glyphosate. Although glyphosine and glyphosate have been reported to be more effective sucrose enhancers than ethephon or mefluidide in whole stalk analysis (Teshima and Osgood, 1977), treatment differences were not statistically significant in this experiment. Increases observed in the sucrose content of internodal tissue at 4 weeks after treatment application indicated a much larger response to exogenous plant growth regulator application than those observed by traditional whole stalk analysis (Table 4). Ethephon, glyphosine and mefluidide showed increases of 70.3%, 37.2% and 15.4% respectively over control in the experiment. The largest increase in sucrose content of immature internodal tissues was caused by glyphosate (75.7%) at 4 weeks after application. These observations are consistent with reports that increases in sucrose content due to plant

growth regulator application are greater in the upper portion of the sugarcane stalk (Tianco and Gonzales, 1980; Clowes, 1980).

L 62-96 is classified as an early maturing sugarcane variety (Richard et al., 1978). No significant increases over control in sucrose percent and CRS/T were observed after treatment application. This was consistent with observations in Part I, that is, the early maturing variety CP 65-357 did not show significant increases in sucrose percent and CRS/T after glyphosate application. This again suggests that a relationship between varietal maturity patterns and plant growth regulator response could exist, however, further experimentation would be necessary to confirm this.

The plant growth regulators examined in this experiment increased sucrose content of immature internodal tissue, but differences in sucrose percent and CRS/T measured by whole stalk analysis were not significant. Using immature internodal tissue analysis may, therefore, overestimate the impact of growth regulators on overall sucrose percent and CRS/T in the whole plant.

PART III. The Effect of Glyphosate on Endogenous Reducing Sugar Levels in Immature Internodal Tissues of Sugarcane.

INTRODUCTION

Endogenous levels of glucose and fructose may represent up to 5% of the fresh weight of immature storage parenchyma tissue in sugarcane (Glasziou and Gayler, 1972). These concentrations are higher than levels found in more mature tissues and together with sucrose, are virtually the only storage sugars in sugarcane. Generally, a decline in reducing sugar concentration in tissues treated with growth repressing plant growth regulants has been reported in sugarcane (Maretzki et al., 1976; Maretzki and Dela Cruz, 1979). However, no attempt has been made to examine endogenous glucose and fructose levels separately.

Models of sucrose accumulation in sugarcane have been reviewed (Glasziou and Gayler, 1972; Giaquinta, 1980). These models postulate that active transport of sucrose into storage parenchyma cells begins with the apoplastic hydrolysis of sucrose into glucose and fructose by a soluble acid invertase (α-D-glucopyranosyl-B-D-fructofuranosidase) (pH optimum 5.0-5.5) in expanding internodes (Sacher et al., 1963). Another acid invertase bound to the cell wall is present in these tissues (pH optimum 3.4-3.8 - Hawker and Hatch, 1965). These authors reported that the soluble acid invertase was virtually

absent in mature tissues, but the bound enzyme remained active. Movement into the symplast is in the form of hexose sugars which are phosphorylated in the cytoplasm and sucrose phosphate synthesized (Hawker and Hatch, 1966). It has been postulated that sucrose phosphate traverses the tonoplast mediated by a carrier mechanism, and sucrose is released into the vacuole (Hawker and Hatch, 1965). An acid invertase is presumed to be located in the vacuole of immature storage parenchyma tissue (Slack, 1965). This enzyme is found in high concentrations in immature internodal tissues and disappears soon after cell expansion is complete. A neutral invertase (pH optimum 7) is postulated to regulate storage in mature internodal tissues (Glasziou and Gayler, 1972). However, the mechanism of this regulation is not fully understood.

Glyphosine applied to whole plants has been shown to decrease total acid invertase activity in immature storage tissues in sugarcane (Alexander, 1976). The author also reported no effect of glyphosine on acid invertase in vitro up to a 10,000 ppm concentration. It was postulated that the effect of glyphosine on acid invertase activity was probably not direct. Glucose has been reported to decrease vacuolar acid invertase activity in tissue slices of sugarcane (Glasziou et al., 1966). By comparing the effect of actinomycin D and glucose on invertase activity, it was postulated that glucose increased the rate of

destruction of mRNA required for invertase synthesis. Also, a decline in total reducing sugar concentration coincided with increased acid invertase activity in immature internodal tissues (Glasziou et al., 1966). However, no attempt was made to determine if this reduction in reducing sugar concentration was due to a decline in either glucose or fructose or both sugars simultaneously.

The objective of this experiment was to determine if a reduction in endogenous glucose or fructose levels would be observed in immature internodal tissue of sugarcane after glyphosate application.

MATERIALS AND METHODS

Plant Tissue. The experimental design, method of treatment application and sampling were conducted as described in Part I. The varieties NCo 310, CP 61-37 and CP 65-357 were selected to examine the effect of glyphosate at .45 kg ae/ha on endogenous reducing sugar levels. Reducing sugars were analyzed by the method of sucrose analysis described in Part I and are reported as mg of glucose or fructose per gram dry weight of immature internodal tissue.

Statistical Technique. Response equation estimation (Tables 5 and 6) and area comparisons made between glyphosate treated and untreated sugarcane are as described for sucrose accumulation in Part I and Appendix II. Data collected in the 1978 and 1979 experiments were used in the analysis. Correlations between sucrose content (Part I) and glucose and fructose levels in immature internodal tissues were also calculated. An α level of 0.05 was used in evaluating all statistical comparisons.

RESULTS

The glucose concentrations of glyphosate treated and untreated immature internodal tissues are shown for the varieties CP 65-357, CP 61-37 and NCo 310 in Figures 5, 6 and 7 respectively. Means observed for all treatments and varieties are shown in Appendix I for glucose (Tables 14 and 15) and fructose concentrations (Tables 17 and 18) in the 1978 and 1979 experiments. The responses of glucose concentration with time for all varieties and both treatments were defined by quadratic equations (Table 5). Analysis of variance tables for glucose and fructose concentrations are shown in Appendix I (Tables 13 and 16). Immature internodal tissues of CP 65-357 showed a decline in glucose concentration with time (Figure 5). Glyphosate treated tissues showed a greater decline in glucose concentration when compared to control throughout the experiment. Maximum difference between the two treatments appeared to occur 7 weeks after glyphosate application. Glucose concentrations also declined in the varieties CP 61-37 and NCo 310 (Figures 6 and 7 respectively). The decline in glucose concentration was more rapid in glyphosate treated tissues of CP 61-37 during the first three weeks after application (Figure 6). The decline in glucose content apparently ceased through the final sampling dates

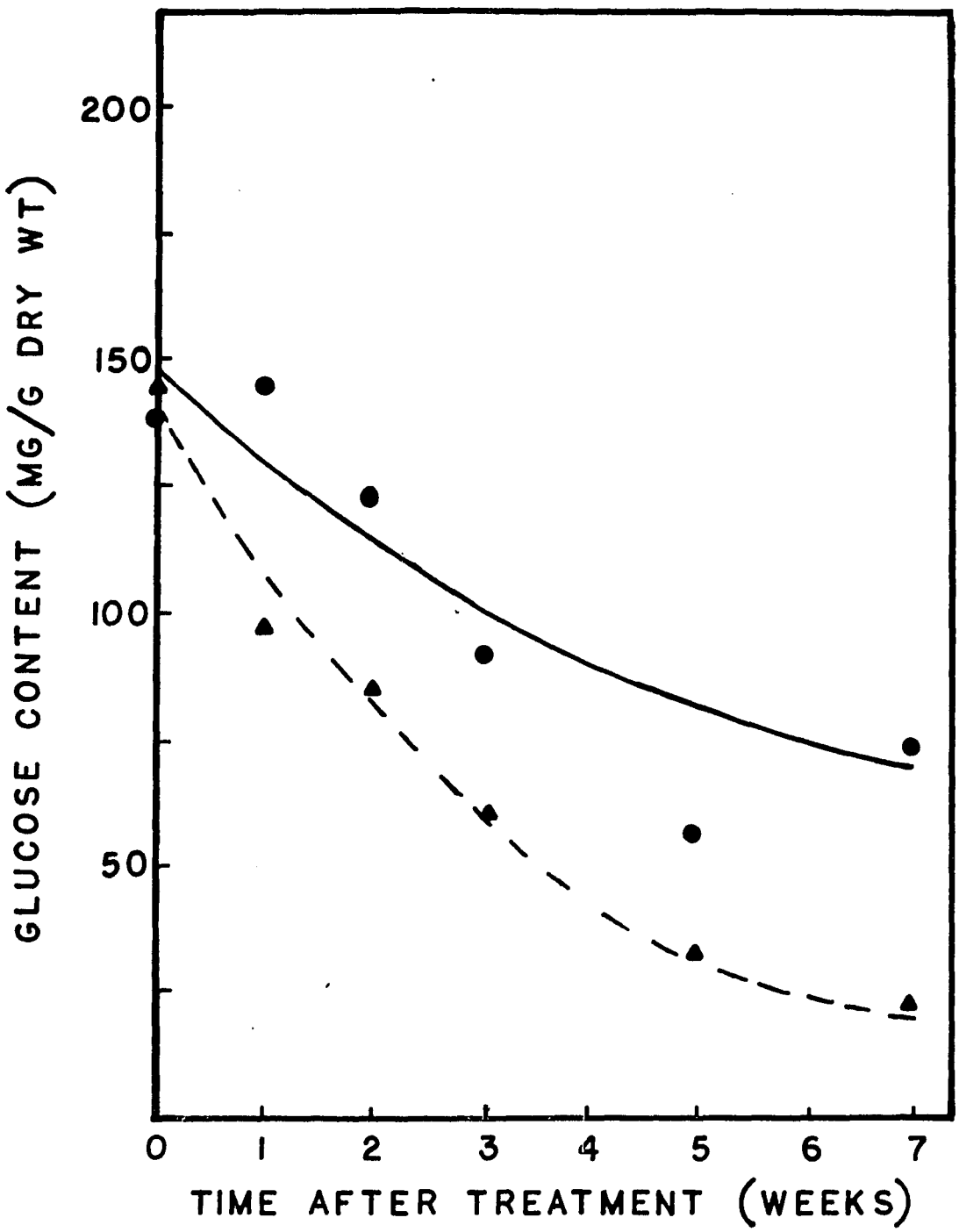

Figure 5. Average glucose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) CP 65-357 after treatment application.

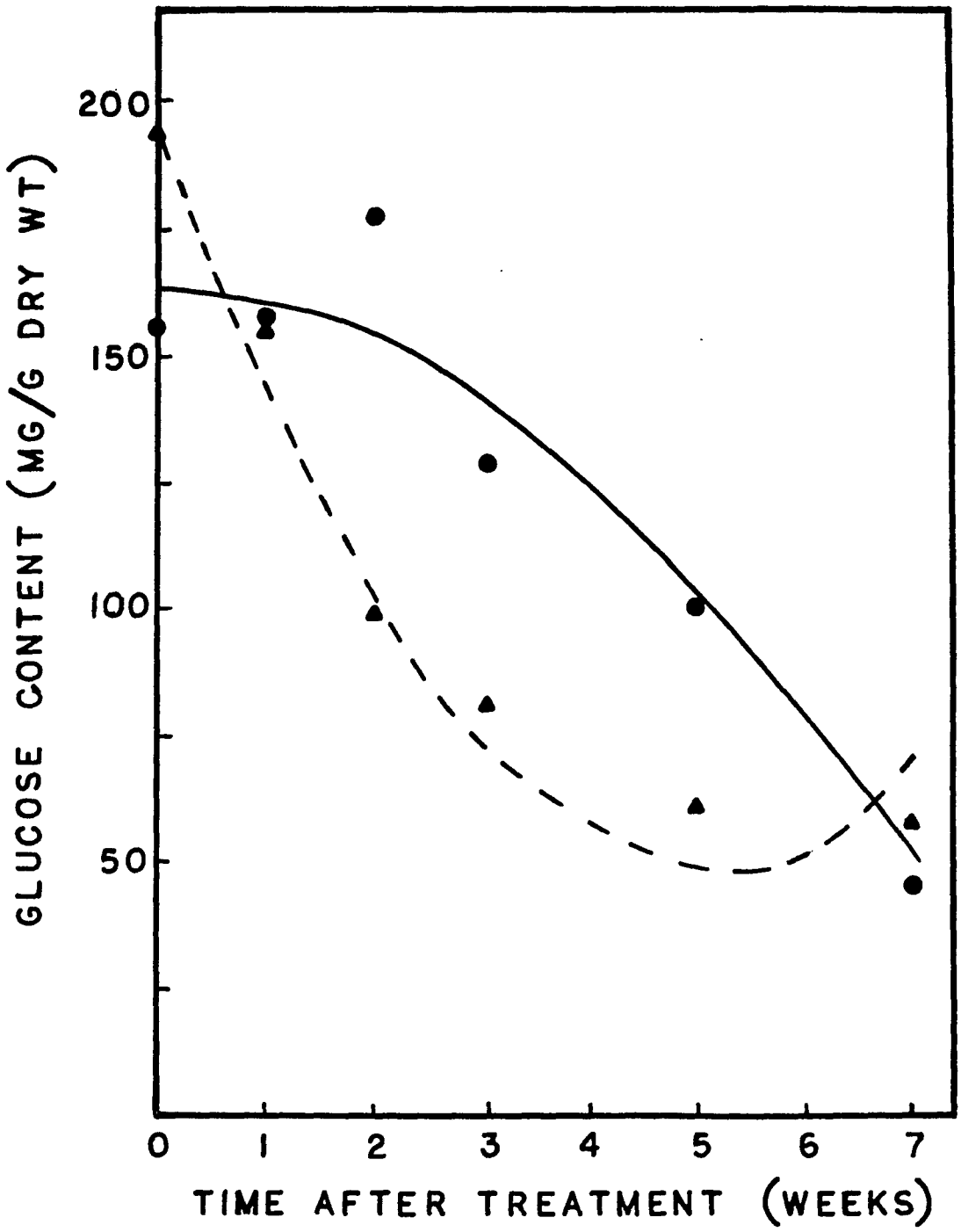

Figure 6. Average glucose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) CP 61-37 after treatment application.

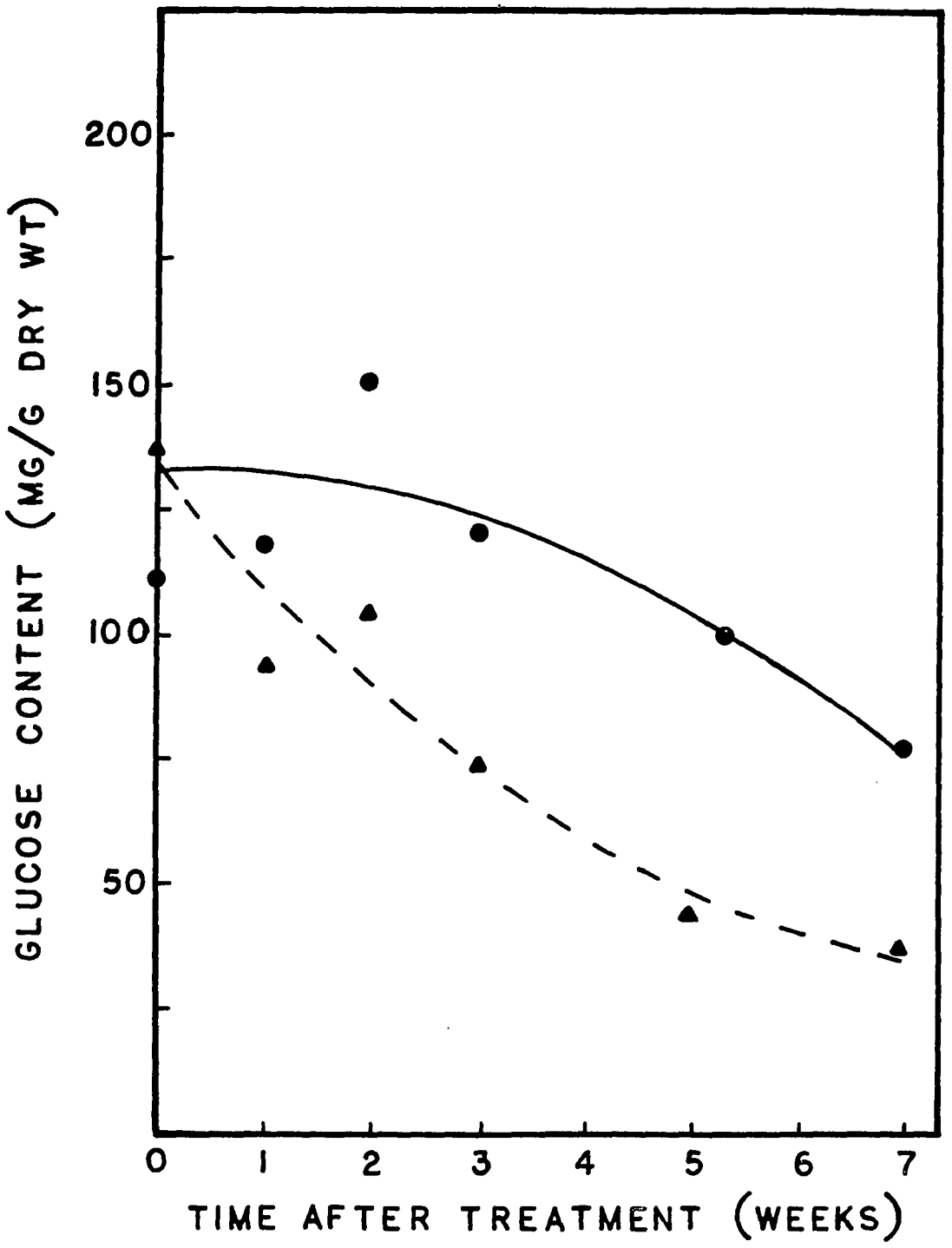

Figure 7. Average glucose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) NCo 310 after treatment application.

Table 5. Estimated response equations for glucose concentration (mg/g dry wt) of immature internodal tissues, x = weeks after glyphosate application.

Variety	Treatment	
	Control	Glyphosate
CP 65-357	$148.09 - 18.87x - 1.08x^2$	$139.22 - 33.29x + 2.30x^2$ *
CP 61-37	$164.74 - 1.69x - 2.12x^2$	$199.57 - 60.27x + 6.01x^2$ *
NCo 310	$133.16 + 0.45x - 1.26x^2$	$132.70 - 24.52x - 1.50x^2$ *

* Significantly different from control at the $\alpha = 0.05$ level.

of the experiment in glyphosate treated tissues of this variety. Glucose concentration in glyphosate treated NCo 310 also declined more rapidly than control after treatment application (Figure 7). The levels of glucose content in immature internodal tissues of all 3 varieties was significantly lower than control in this experiment (Table 5).

The amount of fructose extracted from immature internodal tissues declined through the course of the experiment in all varieties (Figures 8, 9 and 10). Also, the decline in fructose concentration was more rapid in glyphosate treated tissues as shown by the quadratic response curves estimated (Table 6). In each variety, a rapid decline in average fructose concentration was observed through the first 3 weeks after glyphosate application (Figures 8, 9 and 10). Beyond this point average fructose concentrations appeared to level off in glyphosate treated tissues. Fructose concentration declined at a slower rate in untreated tissues of all 3 varieties throughout the experiment. Differences in fructose content between glyphosate treated and untreated immature internodal tissues of all varieties were declared significant (Table 6).

Correlation coefficients between sucrose content and glucose and fructose content are shown in Table 7. Significant correlations were observed in glyphosate treated and

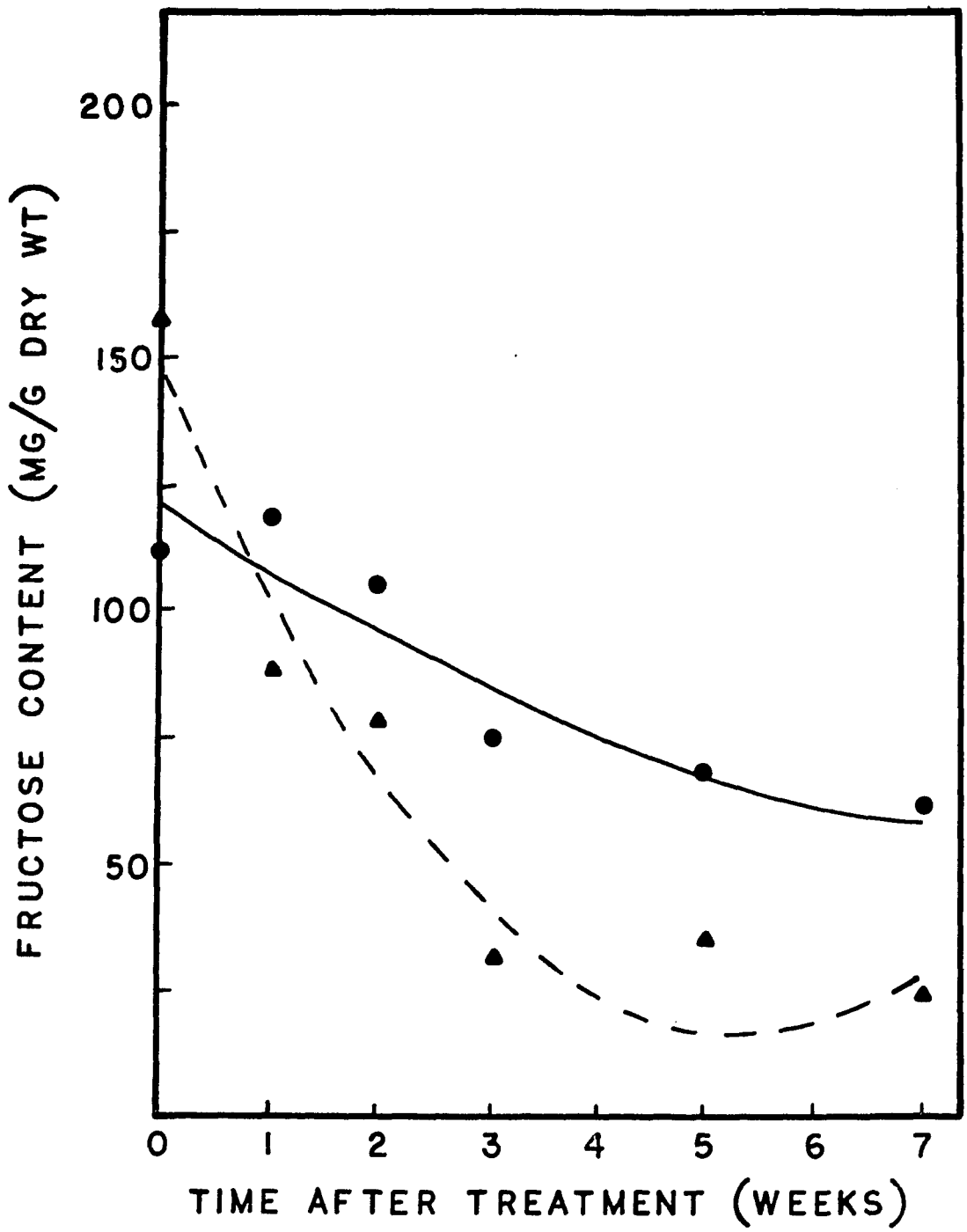

Figure 8. Average fructose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) CP 65-357 after treatment application.

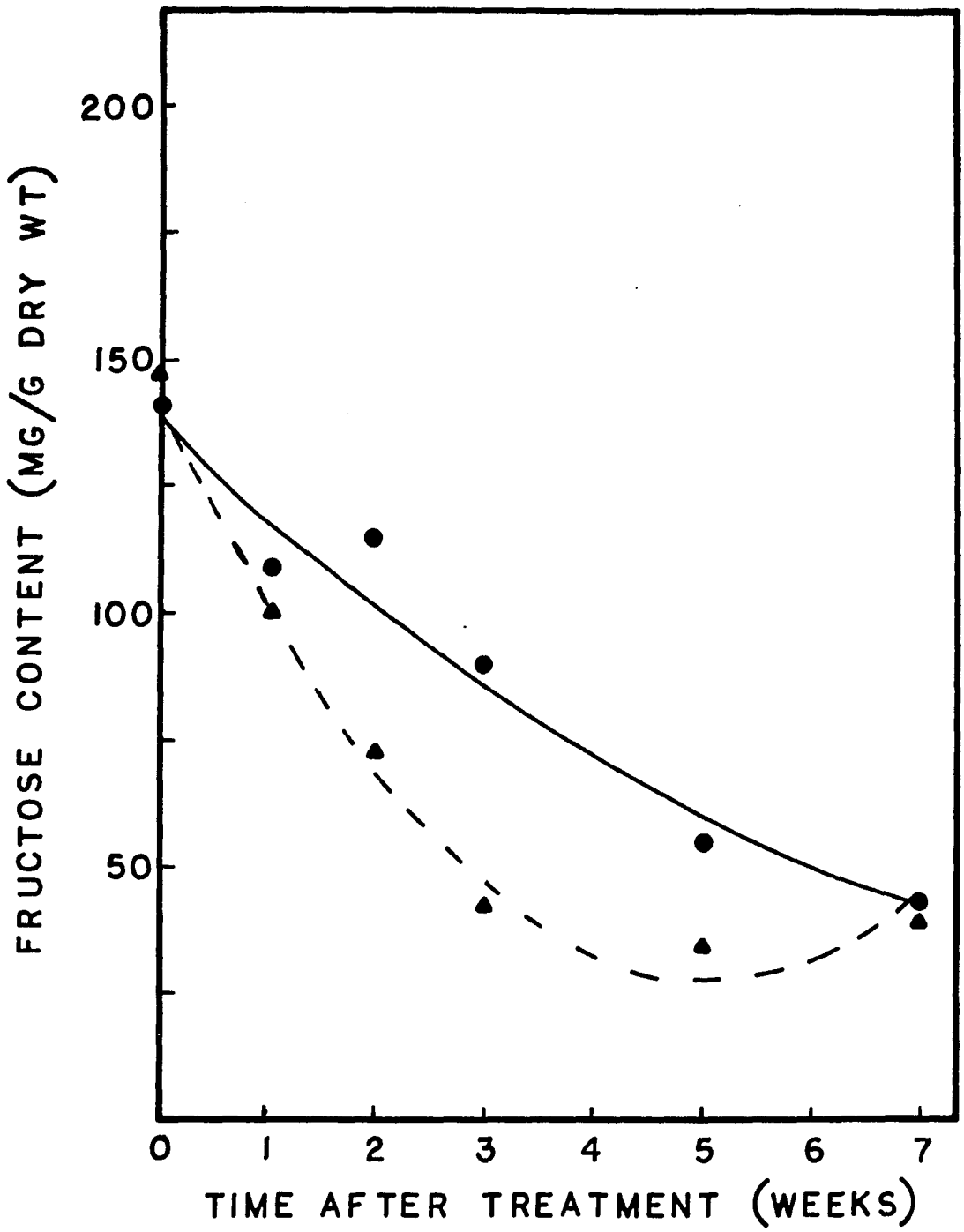

Figure 9. Average fructose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) CP 61-37 after treatment application.

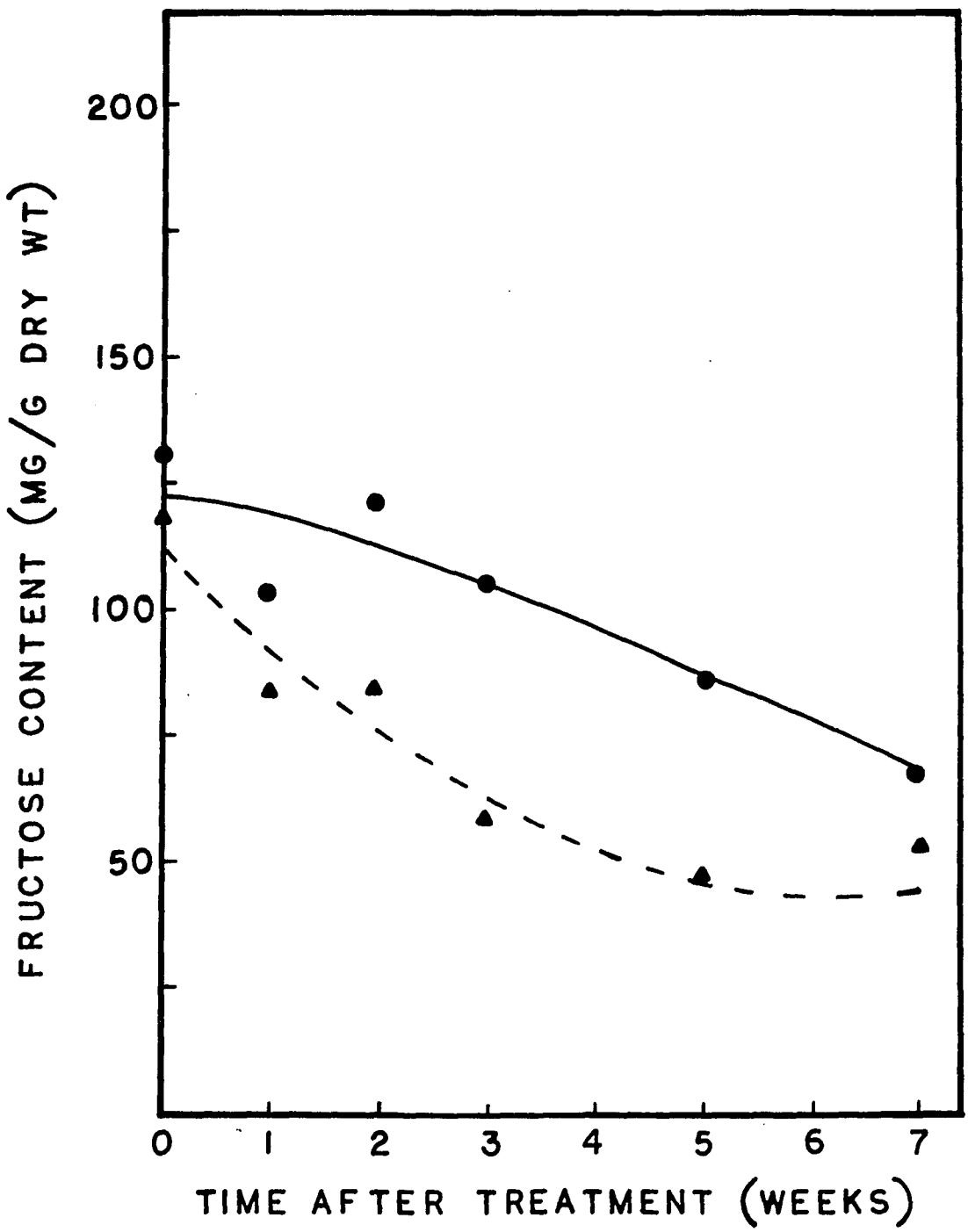

Figure 10. Average fructose concentration of immature internodal tissue of glyphosate treated (- - -, ▲) and untreated (——, ●) NCo 310 after treatment application.

Table 6. Estimated response equations for fructose concentration (mg/g dry wt) of immature internodal tissues, x = weeks after glyphosate application.

Variety	Treatment	
	Control	Glyphosate
CP 65-357	$120.62 - 13.91x + 0.70x^2$	$147.54 - 47.43x + 4.32x^2$ *
CP 61-37	$137.26 - 18.67x + 0.73x^2$	$146.05 - 47.04x + 4.70x^2$ *
NCo 310	$124.36 - 5.74x - 0.32x^2$	$113.40 - 22.12x + 1.73x^2$ *

* Significantly different from control at the $\alpha = 0.05$ level.

Table 7. Correlation coefficients between sucrose content and glucose and fructose content of immature internodal tissues.

	Sucrose Content[1/]					
	CP 65-357		CP 61-37		NCo 310	
	C[2/]	G[3/]	C	G	C	G
glucose content[1/]	-0.4294*	-0.8148*	-0.5753*	-0.7863*	-0.4232*	-0.7704*
fructose content[1/]	-0.4250*	-0.7927*	-0.4838*	-0.7388*	-0.2989*	-0.7765*

[1/]data expressed in mg/g dry weight

[2/]C = control.

[3/]G = glyphosate.

*Significant at the $\alpha = 0.05$ level under Ho: RHO = 0.

untreated immature internodal tissues of all varieties tested. In general correlation coefficients were higher in glyphosate treated tissues than in untreated tissues of all varieties tested (Table 7).

DISCUSSION

A reduction in both glucose and fructose levels occurred in glyphosate treated and untreated tissues of all varieties examined in this experiment (Figures 5-10). Reductions in both glucose and fructose concentrations in tissues of glyphosate treated varieties were significant when compared to control (Tables 5 and 6). The reductions in glucose and fructose concentration observed occurred simultaneously with increased sucrose content observed after glyphosate application (Part I). A significant negative correlation between sucrose content and glucose and fructose content was observed in glyphosate treated and untreated immature internodal tissues of the varieties examined in this experiment (Table 7). Correlation coefficients for sucrose content and both glucose and fructose concentration were higher for glyphosate treated tissues of all varieties examined. This would be expected since an increase in sucrose content over control (Part I) and decline in both glucose and fructose below control levels was observed in the experiment. The reductions in reducing sugar concentrations observed here are consistent with a previous report (Maretzki and Dela Cruz, 1979).

These observations in all likelihood reflect a shift in source-sink relationships within the sugarcane plant

after glyphosate application as has been inferred with glyphosine (Alexander, 1976). Reductions in reducing sugar concentration and increases in sucrose content could be due to the action of glyphosate in the vegetative apex as a growth repressant (Takahashi, 1976; Dill et al., 1980). By curtailing meristematic activity and thus reducing the demand for carbohydrate for processes associated with vegetative growth, photosynthetic product could be shifted to storage in the form of sucrose. Reductions observed in total soluble acid invertase activity of immature internodal tissues after glyphosine application (Alexander, 1976) could be the result of a decline in soluble acid invertase activity which is postulated to function in remobilization of stored sucrose to meet the carbohydrate demands of vegetative growth. This would be consistent with the observation that the efficacy of growth repressants in increasing sucrose content is enhanced when conditions for maximum photosynthetic activity are maintained after application (Osgood and Teshima, 1980). Furthermore, turnover rates of existing sucrose were diminished after glyphosate application (Hilton et al., 1980).

It has been postulated that glucose can effect an increase in the rate of destruction of mRNA necessary for the synthesis of soluble acid invertase (Glasziou et al., 1966). Levels of glucose of 0.05 and 0.11M exogenously

applied to immature sugarcane tissue slices decreased levels of invertase activity. The authors also reported that a decline in endogenous reducing sugar levels coincided with increases in soluble acid invertase activity. The observed reduction in glucose and fructose concentration in this experiment, coupled with increased sucrose content due to glyphosate application (Part I), would seem consistent with the above observations. However, whether or not reductions in reducing sugar concentrations due to glyphosate activity affect invertase synthesis are beyond the scope of this discussion.

The reductions observed in endogenous glucose and fructose concentrations after glyphosate application in these experiments are consistent with previous reports (Maretzki and Dela Cruz, 1979). It also seems that both glucose and fructose levels are affected by glyphosate application, and no differential partitioning occurs between the two reducing sugars after treatment.

PART IV. Sucrose Uptake by Immature Storage Tissue Slices of Sugarcane as Affected by Glyphosate

INTRODUCTION

The kinetics of sugar uptake in immature sugarcane storage tissues were originally investigated by Bieleski (1960a and 1960b). Sugarcane tissue slices removed sucrose and glucose from a bathing medium against a concentration gradient. Models describing the mechanism of sucrose accumulation have been reviewed (Glasziou and Gayler, 1972) and suggest that sugar uptake in immature internodal tissues is the result of an active process. Immature sugarcane storage tissues contain a soluble apoplastic acid invertase (pH optimum 5.0-5.5) which is virtually absent in mature internodes where cell expansion, and, hence, internode elongation is complete (Hawker and Hatch, 1965). A vacuolar acid invertase is also present in expanding internodes but disappears after cell expansion is complete (Glasziou and Gayler, 1972). An acid invertase is also present and bound to the cell wall in immature and mature internodal tissues (Hawker and Hatch, 1965).

Sucrose uptake studies in which immature internodal tissue slices have been used in evaluating uptake rates indicated significant clonal differences (Owaru, 1977). Further, clones reported to have higher rates of sucrose uptake as shown in this system were higher in sucrose

content. Little is known of the effect of exogenously applied plant growth regulators on sucrose uptake in immature tissues. Alexander (1976) has reported that glyphosine decreased the activity of soluble acid invertase in immature storage tissues of treated plants. However, it was also indicated that the effect of glyphosine on soluble acid invertase activity was not direct.

The objective of this study was to examine the effect of foliar applications of the plant growth regulator glyphosate on the uptake of sucrose from an external medium by immature storage tissue slices of sugarcane. Varieties representing a range of response to glyphosate were selected for this experiment. It was postulated that increases observed in sucrose content with time would be reflected in sucrose uptake rates measured in immature internodal tissue slices.

MATERIALS AND METHODS

Experimental Design. The experimental design, method of treatment application and sampling were as described in Part I. The sugarcane varieties CP 65-357, CP 61-37 and NCo 310 were selected to examine sucrose uptake rates as affected by glyphosate.

Statistical Technique. The analysis utilized was the same as described in Part I. Area comparisons were constructed for each of the varieties examined (Appendix II) as reported in Part I.

Sucrose Uptake. Estimation of sucrose uptake was accomplished by incubation of immature internodal tissues in sucrose solutions as previously described (Bieleski, 1960a; Owaru et al., 1977). Internode +3 was removed from each stalk and sectioned as described in Part I. A 5 g sub-sample was taken from each sample of sectioned immature internodal tissue. The tissue was rinsed after weighing for 8-9 hours in 5 changes of tap water to remove free space sugars as previously reported (Bieleski, 1960a; Bieleski, 1960b). The tissue was then transferred to a 20 ml vial to which 10 ml of an 0.5% sucrose solution was added. The solutions were aerated and 0.5 ml aliquots taken from each vial at 4, 8 and 12 hours after the onset

of incubation. Time intervals were chosen for sampling after the linearity of this response with time had been demonstrated (Appendix I - Figure 2) as previously reported (Bieleski, 1960a). The aliquots were analyzed using the LC system under the operating conditions described in Part I. Rates of sucrose uptake were calculated from the difference in sucrose concentration of the bathing medium at 4 and 12 hours after the onset of incubation. Calculations for this variable are shown in Appendix III. Data are reported as the rate of sucrose uptake in mg per g fresh weight of tissue per hour.

Sucrose Content. A 20g subsample of immature internodal tissue was extracted with ethanol as described in Part I to determine sucrose concentration. Data are reported as mg sucrose per g fresh weight of internodal tissue.

RESULTS

The response of sucrose uptake rates (mg/g fresh weight per hour) and sucrose content (mg/g fresh weight) with time for the immature internodal tissues of CP 65-357 is shown in Figure 11. Analysis of variance tables from which statistical comparisons were constructed are shown in Appendix I for sucrose uptake rates (Appendix Table 17) and sucrose content (Appendix Table 20). Means from experiments conducted in 1978 and 1979 for sucrose uptake rates (Appendix Tables 18 and 19) and sucrose content (Appendix Tables 21 and 22) are also presented. The quadratic response estimated for glyphosate treated and untreated tissues (Table 8) indicated a decline in the sucrose uptake rate from the first through the seventh week after treatment application in CP 65-357. The sucrose uptake rate did not appear to decline as rapidly in glyphosate treated tissues (Figure 11). However, statistical analysis indicated that the sucrose uptake rate in glyphosate treated tissues was not significantly higher throughout the experiment (Table 8). The sucrose content of glyphosate treated tissues increased over control throughout the experiment (Figure 11). The largest increase occurred at 7 weeks after glyphosate application and represented

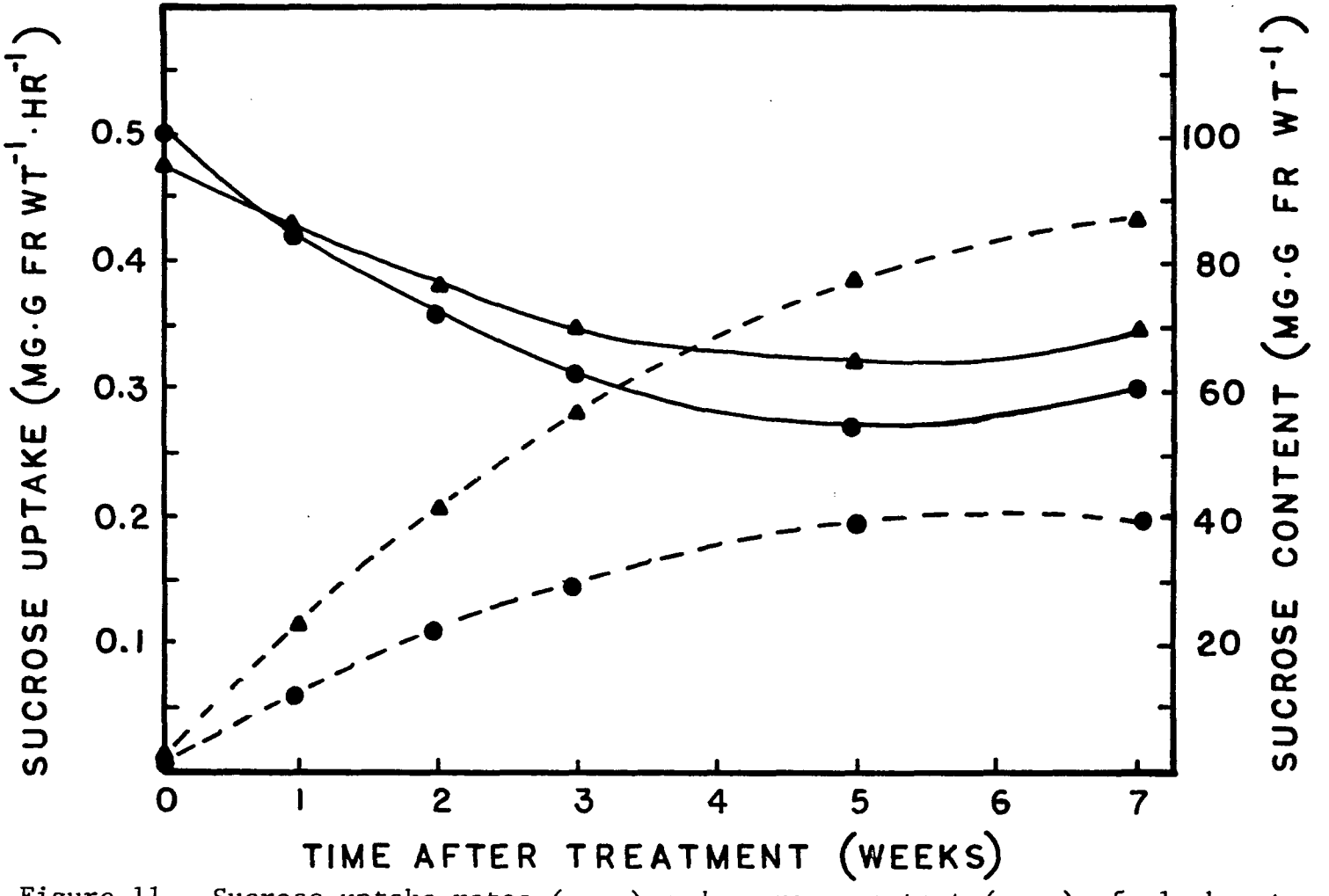

Figure 11. Sucrose uptake rates (——) and sucrose content (- - -) of glyphosate treated (▲) and untreated (●) immature internodal tissue of CP 65-357 after treatment application.

Table 8. Estimated response equations for the sucrose uptake rates (mg/g fresh weight per hour) of immature internodal tissues, x = weeks after glyphosate application.

	Treatment	
Variety	Control	Glyphosate
CP 65-357	$0.500 - 0.091x + 0.009x^2$	$0.474 - 0.060x + 0.006x^2$
CP 61-37	$0.512 - 0.129x + 0.041x^2$	$0.323 + 0.035x - 0.009x^2$
NCo 310	$0.497 - 0.175x + 0.021x^2$	$0.381 - 0.003x - 0.002x^2$*

* Significantly different from control response at the $\alpha = 0.05$ level.

a 120% increase in sucrose content over control. Statistical analysis involving the quadratic response equations indicated that the increase in sucrose content of glyphosate treated tissues was significantly higher than control (Table 9).

The sucrose uptake rates plotted against weeks after treatment application of glyphosate treated and untreated CP 61-37 are shown in Figure 12. An overall decline was also observed in the sucrose uptake rate of both treated and untreated tissues by the response equations estimated (Table 8). The sucrose uptake rate in glyphosate treated tissues did not appear to decline over the initial stages of the experiment, and was apparently higher than observed in untreated tissues from the second to fifth weeks after application (Figure 12). These differences were not declared statistically significant (Table 8). The amount of sucrose extracted from the immature internodal tissues of CP 61-37 was higher in glyphosate treated tissues during the latter stages of the experiment (Figure 12). This increase was significant (Table 9) and most prominent at the fifth and seventh week after glyphosate application.

Sucrose uptake rates in glyphosate treated tissues of NCo 310 were higher than control 2 through 5 weeks after application (Figure 13). A rapid decline in the sucrose uptake rate in untreated tissue slices was observed through the third week of the experiment. As indicated, sucrose

Table 9. Estimated response equations for the sucrose content (mg/g fresh weight) of immature internodal tissues, x = weeks after glyphosate application.

	Treatment	
Variety	Control	Glyphosate
CP 65-357	$1.85 + 11.81x - 0.91x^2$	$0.30 + 23.08x - 1.51x^2$ *
CP 61-37	$6.30 + 3.07x + 0.58x^2$	$16.83 - 1.23x + 1.85x^2$ *
NCo 310	$2.52 + 5.37x - 0.09x^2$	$1.55 + 15.69x - 0.76x^2$ *

* Significantly different from control response at the $\alpha = 0.05$ level.

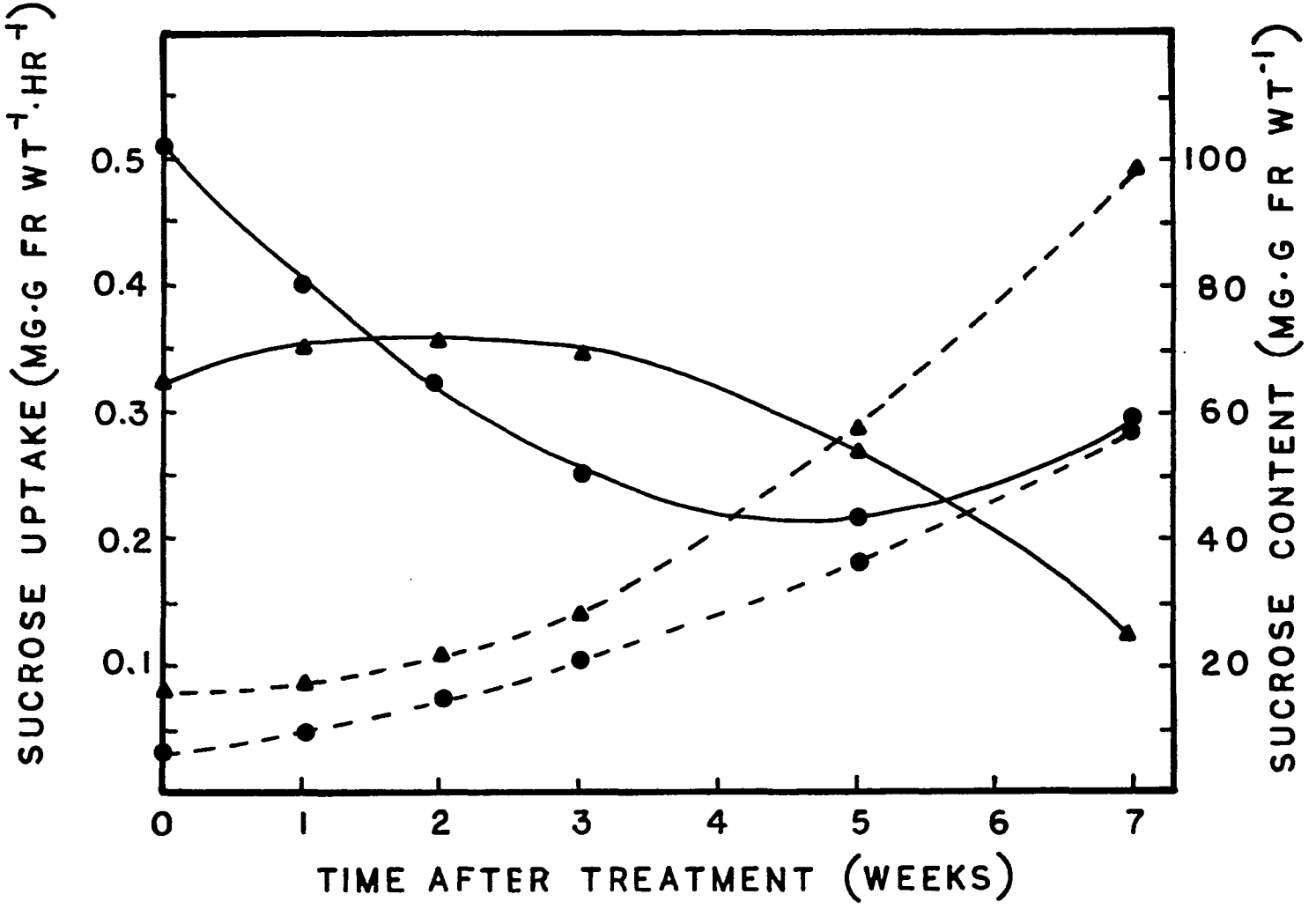

Figure 12. Sucrose uptake rates (——) and sucrose content (– – –) of glyphosate treated (▲) and untreated (●) immature internodal tissue of CP 61-37 after treatment application.

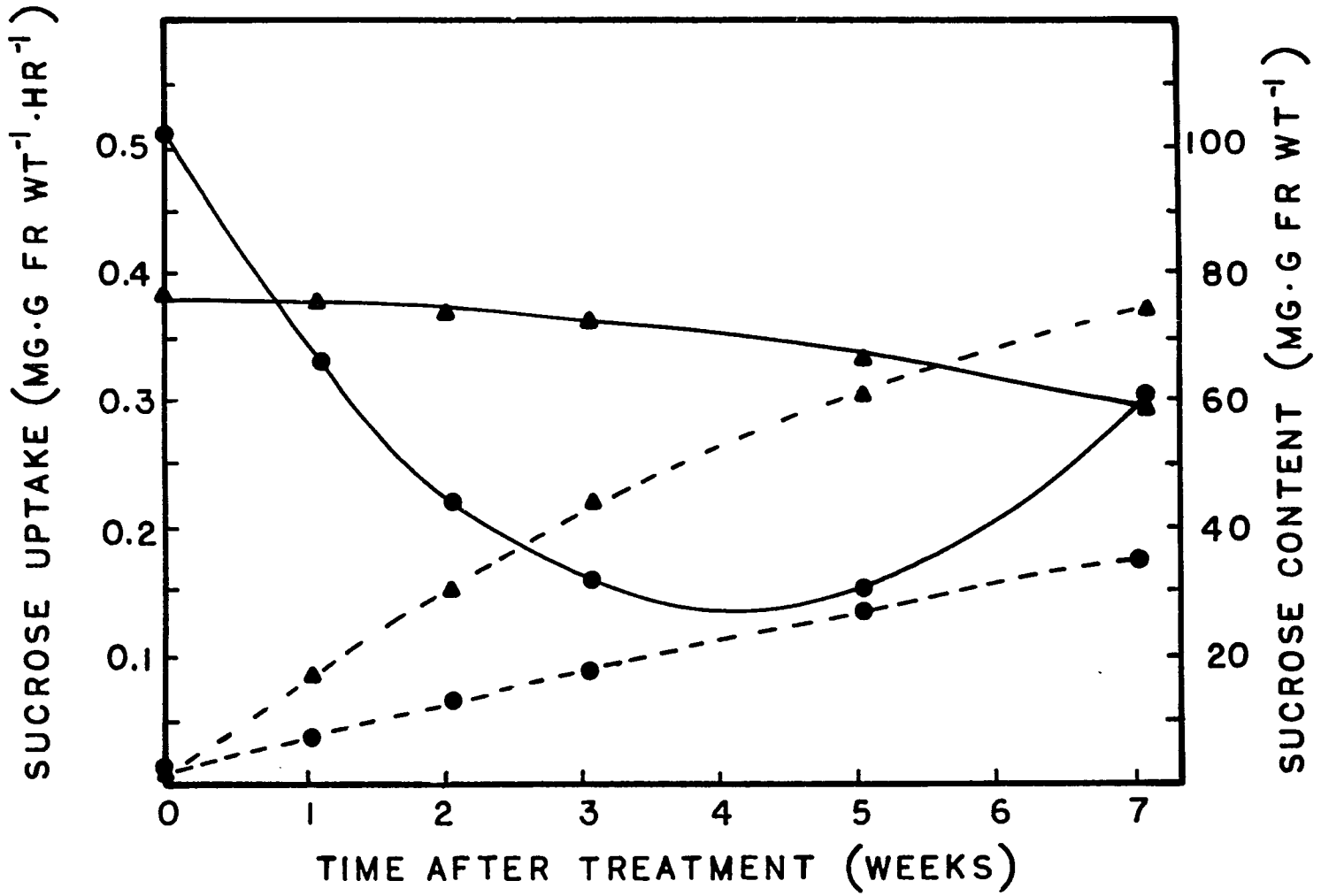

Figure 13. Sucrose uptake rates (——) and sucrose content (- - -) of glyphosate treated (▲) and untreated (●) immature internodal tissues of NCO 310 after treatment application.

uptake rates declined less than control in glyphosate treated tissues (Figure 13). The sucrose uptake rate was significantly higher in glyphosate treated tissues of this variety (Table 8). An increase in the sucrose content of glyphosate treated tissues over control was also observed (Figure 13). This increase was maximum at the 7th week after treatment application and was statistically significant (Table 9).

DISCUSSION

The sucrose uptake rates observed in immature storage parenchyma tissues of sugarcane declined from the first to fifth week of sampling in all varieties tested (Figures 11-13). This decline was consistent with a rise in the sucrose content in these same tissues. It is likely that the increase in sucrose content in both glyphosate treated and untreated tissues was a reflection of internode development. That is, as time progressed in these experiments, environmental conditions became less favorable for vegetative growth and more favorable for sucrose accumulation (Legendre, 1975). The increase in sucrose content of these tissues would then be a reflection of the fulfillment of the sucrose storage capacity of the internode. The decline in the sucrose uptake rate across this same time interval might then be a reflection of decreased soluble apoplastic acid invertase and vacuolar acid invertase activity with development of the internodal tissues as has been reported (Hawker and Hatch, 1965; Glasziou and Gayler, 1972). The increase in sucrose content could then be a reflection of a greater amount of sucrose being stored due to decreased demand for carbohydrate for growth. Despite an overall decline in sucrose uptake rates this change in the partitioning of sucrose between storage and utilization

would then result in an overall increase in the sucrose content of the tissues.

The quadratic response of sucrose content (mg/g fresh weight) with time observed in this experiment (Figures 11-13) was very similar to those observed in sucrose percent and CRS/T as estimated by whole stalk analysis (Osgood, 1979). The relationship between the sucrose content of glyphosate treated and untreated tissues was also similar to those described in whole stalk sugar yield analysis (Martin et al., 1980). This would indicate that sucrose responses to glyphosate application as monitored in sugar yield are reflected in the immature storage parenchyma tissues of sugarcane.

The sucrose uptake rates of glyphosate treated tissues generally appeared to decline less rapidly than those of untreated tissues during the earlier stages of the experiment (Figures 11-13). This effect was significant only in NCo 310 (Figure 13). The sucrose uptake rate measured in slices of glyphosate treated tissues declined only slightly when compared to untreated tissues. An accompanying increase in sucrose content over control was also observed during this period (Figure 13). However, it can by no means be inferred that the direct mechanism of action of glyphosate is to increase the rate of sucrose accumulation in sugarcane. The report that glyphosate functions as an inhibitor of an enzyme of the shikimic

acid pathway (Steinrucken and Amrhein, 1980), and reports that glyphosate failed to enhance the sucrose content of isolated sugarcane cells in suspension culture (Maretzki et al., 1978) would support the contention that increases in the rate of sucrose uptake would not be a primary effect of the compound.

Environmental conditions which enhance sucrose levels in sugarcane are generally unfavorable to vegetative growth. Low temperature, soil water, and nitrogen availability effect an increase in the sucrose content of sugarcane (Legendre, 1975). High incident sunlight has also been positively correlated with increased sucrose content in sugarcane (Legendre, 1975). Glyphosate also increases sucrose content in sugarcane accompanied by reductions in the vegetative growth rate (Dill et al., 1980). Environmental conditions unfavorable to vegetative growth reduce the efficacy of glyphosate as a plant growth regulant in sugarcane (McCatty, 1980). It would then seem that glyphosate induces conditions which are conducive to increases in sucrose content that would occur at a more gradual rate under favorable environmental conditions. Similarities in the nature of treated and control responses of sucrose content with time in this experiment (Figures 11-13), and those reported in sucrose percent and CRS/T (Martin et al., 1980) would seem to support this contention.

PART V. Nitrate Reductase Activity in Sugarcane: Relation to Sucrose Content and Response to Plant Growth Regulator Application.

INTRODUCTION

Research on the effect of exogenously applied phytohormones on nitrate reductase (NR) activity has been reviewed in the recent literature (Srivastava, 1980). Very little information is available on the effects of herbicides, fungicides or synthetic plant growth regulants in agronomic species. Exogenous application of glyphosine was not reported to affect *in vivo* leaf NR activity in sugarcane (Dill, 1977). However, indications of the effects of any other plant growth regulants on NR activity in sugarcane have not appeared in the recent literature.

Genetic variation in NR activity has been recognized as important in relation to yield in crop species. Positive correlations were established between NR activity and yield in corn (*Zea mays* L.) in a survey conducted over two seasons (Zieserl and Hageman, 1962). NR activity of the total leaf canopy expressed as a seasonal average was positively correlated with grain protein and grain yield in corn (Deckard *et al.*, 1973). Similar studies have shown significant positive correlations between NR activity and grain protein content in wheat (*Triticum aestivum* L.) (Croy and Hageman, 1970), barley (*Hordeum vulgare* L.) (Tokarev

and Shumnyi, 1976) and yield in sorghum (Sorghum bicolor L.) (Eck et al., 1975). Varietal differences in sugarcane NR activity have been reported (Dill, 1977). However, no attempt was made to correlate NR activity and sugarcane yield in this experiment.

No correlations were established between NR activity and grain protein content in more recent studies with barley (Oh et al., 1980) and wheat (Deckard and Bush, 1978). In the latter study, it was reported that in vivo NR activity of wheat seedlings grown in the field and that of seedlings grown in a growth chamber were not correlated. Similar results were noted in a study which attempted to correlate NR activity and sorghum yield (Eck et al., 1975). In this instance, growth chamber experiments were not indicative of observations made utilizing field grown plants.

This study was undertaken to determine if a relationship could be established between NR activity and sugar yield in sugarcane. A wide range of genotypes known to be different with respect to sugar yield were selected for this purpose. Also, the NR activity of glyphosate treated commercial sugarcane varieties was monitored to detect whether or not changes in sucrose affected by this compound would be reflected in NR activity.

MATERIALS AND METHODS

NR Activity and Plant Growth Regulator Response. The experimental design, method of treatment application, sampling intervals and statistical analysis were as described in Part I. The varieties NCo 310, CP 61-37 and CP 65-357 were selected to examine the effect of glyphosate at 0.45 kg ae/ha on *in vivo* nitrate reductase activity at leaf position +3 (Van Dillewijn, 1952).

NR Activity vs. Yield. Eighteen varieties (Table 2) representing a wide range of sugarcane genotypes were planted in 1.8 x 1.8 m plots in a completely randomized design containing 2 replicates at the St. Gabriel Agricultural Experiment Station. These same varieties were also planted in the greenhouse as single bud segments in flats containing Jiffy Mix Plus (Jiffy Products Co.) in the same design. *In vivo* NR activity was assayed in both field and greenhouse plants at 4 weeks after shoot emergence in leaf position +3 (Van Dillewijn, 1952). Yield data were collected from field plots as previously described (Part I). Correlations between NR activity in field and greenhouse grown seedlings and the yield estimates, sucrose percent of cane, commercially recoverable sugar per ton of cane (CRS/T), and mean stalk weight were made for data collected

over the 1979 and 1980 growing seasons.

NR Assay. The procedure used was that described by Harper and Hageman (1972) and Dill (1977). Leaf lamina were prepared by sectioning. The leaf midrib was stripped out and only one of the remaining halves used. The remaining lamina portion was torn lengthwise by hand into strips 3-5 mm in width. These strips were then cut into 3-5 mm lengths using a paper cutter.

Five g sub-samples from each group of sectioned lamina were placed in a 20 ml vial to which 10 ml incubation media was added. The media contained 0.15 M potassium phosphate buffer pH 7.5, 0.1 M KNO_3 and 0.4% (v/v) triton X-100 surfactant. The vials were then placed in a bell jar and vacuum infiltrated twice for 5 min at -20 mm Hg. Samples were then placed in a Blue M Electric Co. Magni-Whirl constant temperature bath at 32 C and agitated at 40 cycles per minute for the duration of the incubation period.

Aliquots of 0.2 ml were taken from the media at 20 and 80 minutes after initiation of incubation using a 1.0 ml syringe. The aliquots were placed in 8.0 ml test tubes to which 1.8 ml H_2O and 2.0 ml stop reaction solution were added. The stop reaction solution contained: 1.0% (w/v) sulfanilamide reagent in 1.5 N HCl and 0.02% (w/v) N-(1-napthyl)-ethylenediamine dihydrochloride solution mixed in a 1:1 ratio. Tubes were then allowed to stand at room

temperature for a minimum of 20 min for the characteristic red color to develop. Samples were then assayed for absorbance with a Perkin-Elmer model 124 double beam grating spectrophotometer with a Coleman 48 sample programmer and a Perkin-Elmer model 56 recorder. Absorbance was read at a wavelength setting of 540 nm.

A standard curve was determined for converting absorbance readings to μmoles NO_2^- per 4 ml tube by preparing a series of dilutions of a 2×10^{-7} M KNO_2 solution in a 4 ml volume of distilled H_2O and stop reaction solution. Eight dilutions resulted in a concentration range of NO_2^- of 0.01-0.08 μmoles (by 0.01 μmolar increments) per 4 ml in each tube. Four replicates of each concentration were read at a wavelength setting of 540 nm and a linear relationship between NO_2^- concentration and absorbance at 540 nm was observed. A best fit linear equation (μmoles NO_2^- = (Absorbance (540 nm) x .0939) -7.0×10^{-4}) was used in converting absorbance readings to the μmolar concentration in each tube. Nitrate reductase (NR) activity is reported as μmoles NO_2^- produced per g fresh weight per hour and calculated as shown in Appendix III.

RESULTS

NR Activity and Plant Growth Regulator Response. NR activities for glyphosate treated and untreated CP 65-357, CP 61-37 and NCo 310 are shown in Figures 14, 15 and 16 respectively. In vivo leaf NR activity appeared to decline in untreated tissues through the third week of the experiments and ceased beyond that point. Although the quadratic response equations estimated indicated that NR activity in glyphosate treated tissues of CP 65-357 (Figure 14) was higher than control, this difference was not statistically significant (Table 10). This observation was also made in the varieties CP 61-37 (Figure 15) and NCo 310 (Figure 16).

NR Activity vs. Yield. Average sucrose percent, CRS/T, average stalk weight and NR activity of field and greenhouse grown sugarcane seedlings are shown in Table 10. Varieties are ranked from highest to lowest with respect to sucrose percent and CRS/T. Highest average sucrose percent (13.9%) and CRS/T (103.59 kg/ton) were observed in CP 65-357. Highest NR activity was observed in seedlings of US 67-36-4 in field experiments (0.415 μmoles NO_2^- produced per g fr wt per hour) and CP 62-258 in greenhouse grown seedlings (1.511 μmoles NO_2^- produced per g fr

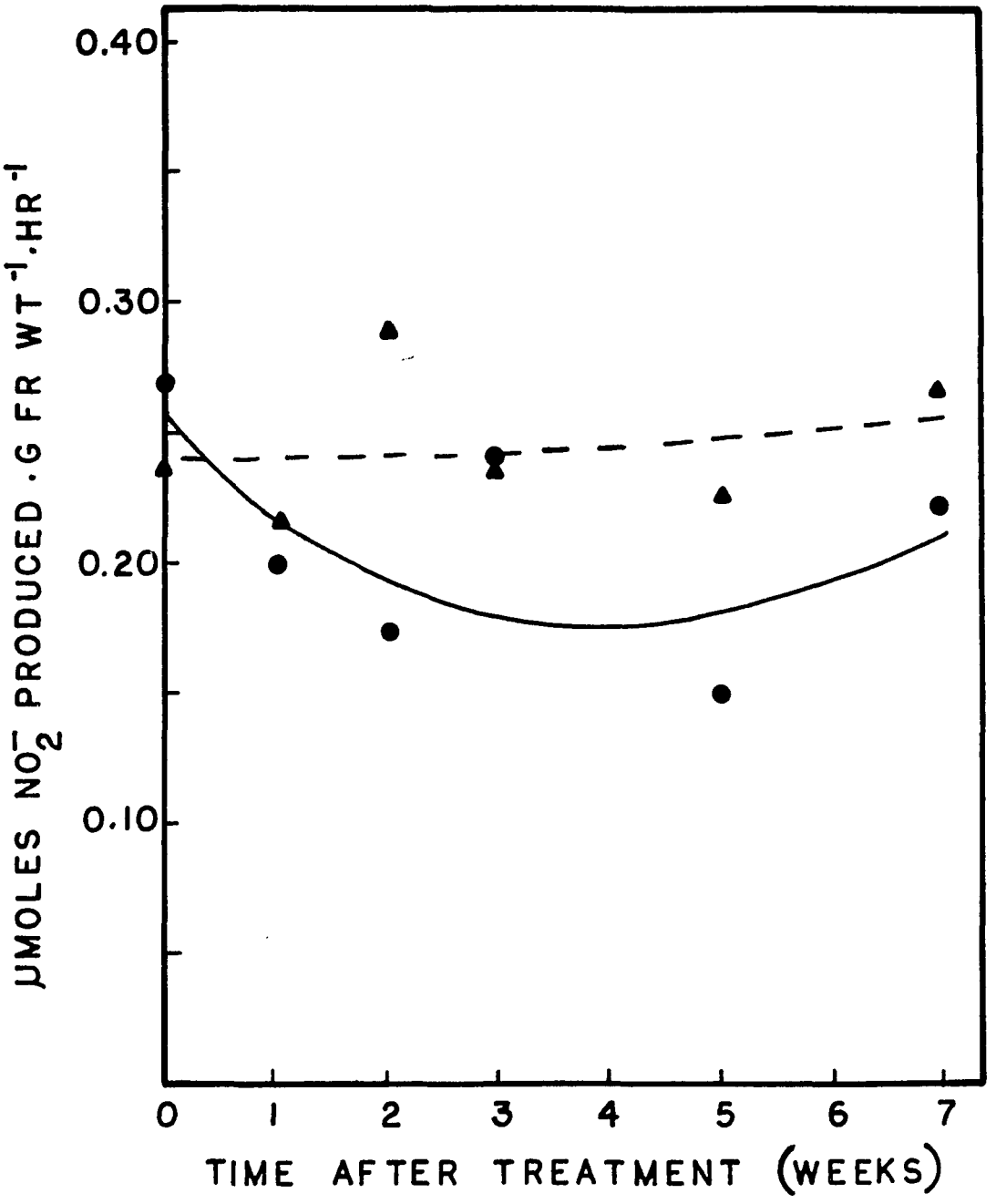

Figure 14. Average NR activity of glyphosate treated (▲) and untreated (●) CP 65-357 after treatment application.

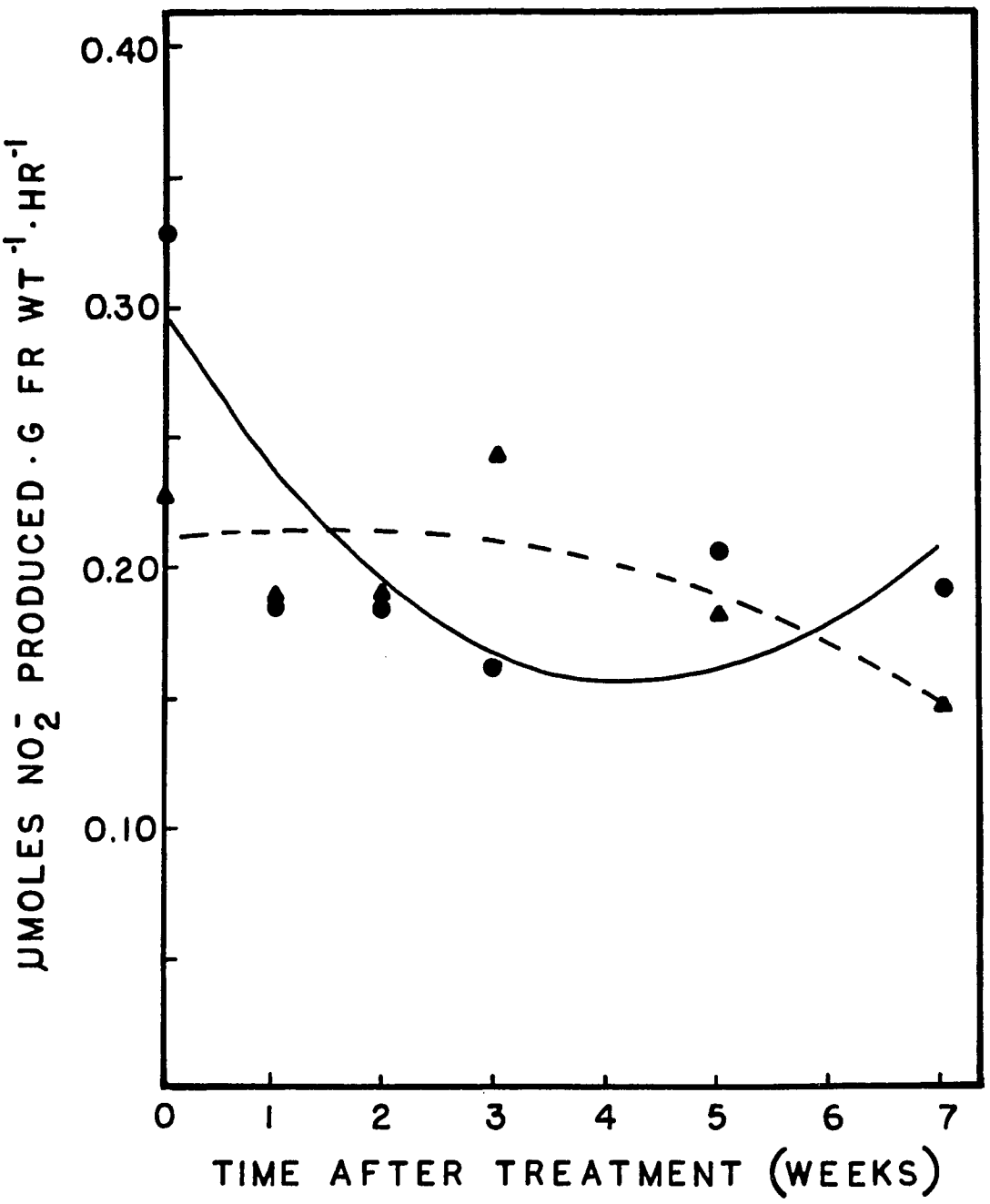

Figure 15. Average NR activity of glyphosate treated (▲) and untreated (●) CP 61-37 after treatment application.

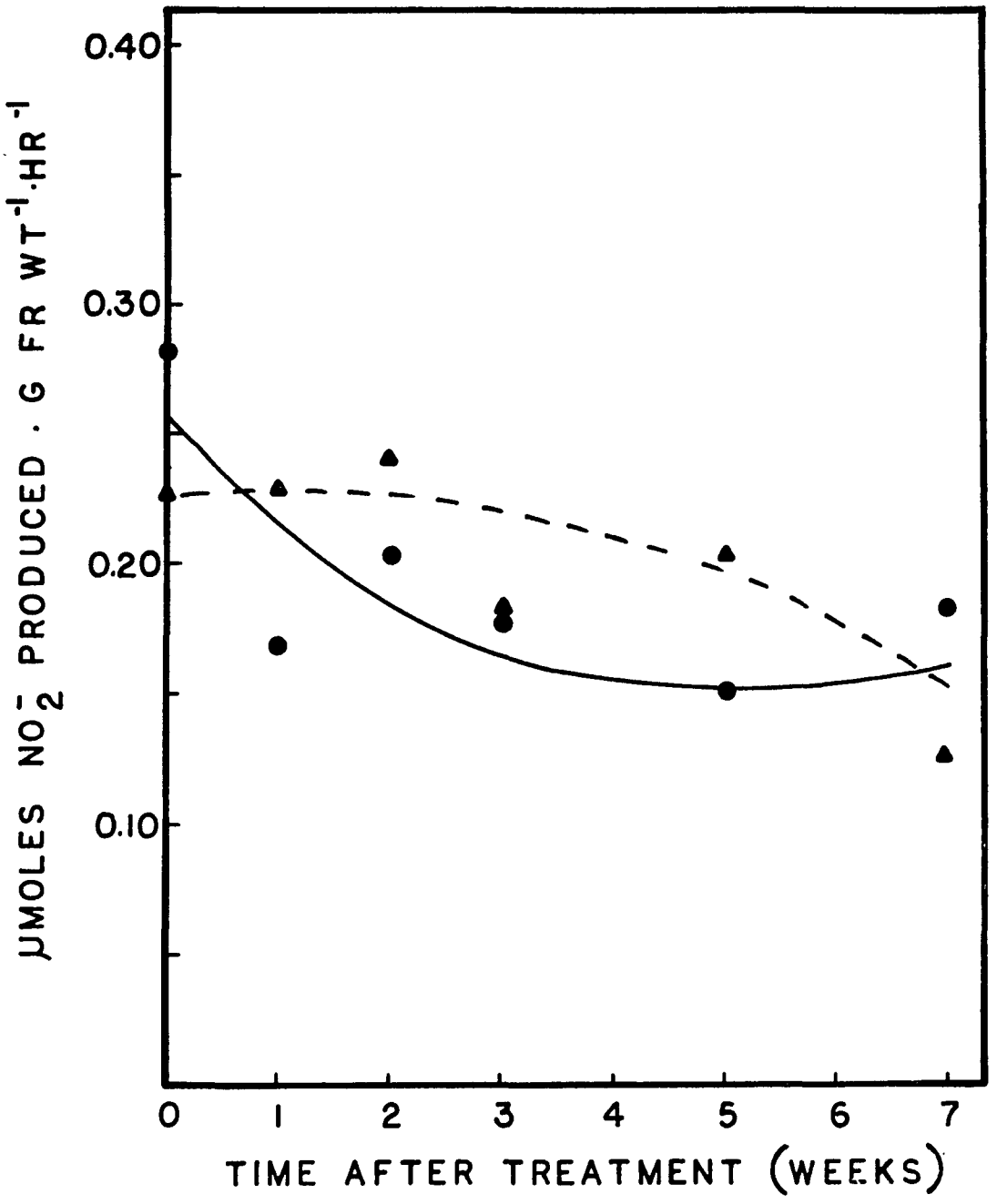

Figure 16. Average NR activity of glyphosate treated (▲) and untreated (●) NCo 310 after treatment application.

Table 10. Estimated quadratic response equations for NR activity, x = weeks after glyphosate application.

Variety	Treatment	
	Control	Glyphosate
CP 65-357	$0.253 - 0.037x + 0.005x^2$	$0.240 + 0.003x - 0.003x^2$
CP 61-37	$0.289 - 0.062x + 0.007x^2$	$0.212 - 0.006x - 0.002x^2$
NCo 310	$0.259 - 0.045x + 0.005x^2$	$0.227 + 0.004x - 0.002x^2$

* Significantly different from control at the $\alpha = 0.05$ level.

wt per hour) (Table 11). The variety CP 77-415 had highest average stalk weight (1.96 kg). No significant correlation between NR activity in field or greenhouse grown sugarcane seedlings and those yield variables measured were established (Table 12). Also, no correlation was established between NR activity measured in field and greenhouse experiments. Differences among varieties were declared significant by analysis of variance for all variables (Appendix I - Table 28).

Table 11. Average sucrose percent, CRS/T, stalk weight and field and greenhouse grown seedling NR activity. Data are expressed as the average of the 1979 and 1980 experiments.

Variety	Sucrose Percent	CRS/T (kg/ton)	Stalk Weight (kg)	NR[1/] (Field)	NR[2/] (GH)
CP 65-357	13.94	103.59	1.37	0.216	0.298
CP 70-321	13.46	72.33	1.28	0.122	0.928
CP 62-258	13.39	70.39	1.00	0.228	1.511
US 67-36-4	12.46	66.07	0.91	0.415	0.808
US 73-80	12.34	63.76	0.89	0.208	0.474
US 72-231	11.28	58.56	0.52	0.111	0.710
NCo 310	11.19	58.34	1.20	0.253	0.889
US 72-166	10.02	50.45	0.54	0.138	1.238
CP 77-415	9.96	50.18	1.96	0.166	0.751
US 74-3	9.86	47.77	0.91	0.402	0.707
US 72-71	9.60	43.70	0.50	0.131	0.939
US 76-9	8.63	40.69	0.55	0.226	0.938
US 76-36	8.61	40.66	0.55	0.279	1.223
US 72-162	8.41	39.14	0.71	0.132	0.652
US 76-10	7.67	32.88	0.48	0.152	1.224
US 66-9	7.03	31.31	0.96	0.276	0.493
US 66-56-9	6.05	23.60	0.91	0.278	0.779
US 72-37	5.56	20.44	0.60	0.178	0.977

1/ μmoles NO_2^- produced per g fresh weight per hour.

Table 12. Coefficients of correlation obtained from the relationships between NR activity (field and greenhouse grown seedlings) and sucrose percent, CRS/T, and average stalk weight data collected in the 1979 and 1980 experiments.

NR Activity[1/]	Average Stalk Weight (kg)	Sucrose Percent	CRS/T (kg/ton)	NR Activity[1/]
Field Grown Seedlings	0.0627	-0.1051	-0.1047	-
Greenhouse Grown Seedlings	-0.0929	0.2446	0.2326	-0.1235

1/ μmoles NO_2^- produced per g fresh weight per hour.

* r value significantly different from zero by Ho: RHO = 0, α = 0.05 level.

DISCUSSION

NR Activity and Plant Growth Regulator Response. The effect of glyphosate on the NR activity of the varieties CP 65-357, CP 61-37 and NCo 310 is shown in Figures 14, 15 and 16 respectively. The increase observed in these experiments due to glyphosate application were not significant in any of the varieties tested (Table 10). No differences in NR activity were observed in a previous study between glyphosine treated and untreated sugarcane leaf tissues (Dill, 1977). In examining those results observed in the previous section, it would seem likely that a relationship between NR activity and sucrose enhancement as affected by plant growth regulants would not be observed. The inverse relationship between vegetative growth and increased sucrose content associated with plant growth regulators in sugarcane might indicate that a negative correlation could be observed between NR activity and sucrose content. However, such a relationship was not observed in this experiment. Since no correlation appears to exist between NR activity and sucrose content in sugarcane, any effect of glyphosate on *in vivo* leaf NR activity would probably not reflect changes in sugar content in sugarcane.

NR Activity vs. Yield. Correlations between NR activity of field and greenhouse grown seedlings and yield data collected from field plots were not established in this experiment (Table 12). Rankings of average sucrose percent and average CRS/T did not coincide with NR activity in these experiments (Table 11). Correlations between NR activity and yield have been established in sorghum (Eck et al., 1975), corn (Deckard et al., 1973) and wheat (Deckard et al., 1977). However, more recent studies have indicated the contrary in barley (Oh et al., 1980) and wheat (Deckard and Bush, 1978). This would indicate that the use of NR activity for predicting ultimate yield in agronomic species is somewhat questionable.

The lack of a consistent relationship between NR activity in field and greenhouse grown seedlings indicated unpredictable variation under different environmental conditions for this variable. Deckard and Bush (1978) reported no correlation between NR activity between field and growth chamber grown wheat seedlings. Growth chamber experiments were also not indicative of field results for NR activity in sorghum (Eck et al., 1975). In the latter study NR activity in field grown sorghum seedlings was correlated with sorghum yield. The failure of NR activity determinations made under the more controlled conditions of greenhouse and growth chamber experiments to relate with

field observations would indicate extreme caution is necessary in interpreting such results.

Varietal differences observed in this experiment with respect to in vivo NR activity have been previously reported in sugarcane (Dill, 1977). This observation is consistent with reports of genetic variation in NR activity in other agronomic species (Zieserl and Hageman, 1962). This observation was expected due to the wide range of genetic material selected for this experiment.

SUMMARY

An increase in sucrose content of immature internodal tissues was observed in all varieties tested after plant growth regulator application (Figures 1-4). The percent difference between plant growth regulator treated and untreated internodal tissue was much greater than that observed in sucrose percent and CRS/T determined by whole stalk analysis 4 weeks after treatment application (Tables 2 and 4). This indicated a greater increase in sucrose occurred in the upper portion of the stalk as was previously reported (Tianco and Gonzales, 1980). Results observed for two varieties suggest that it may be possible to evaluate the sucrose enhancing properties of plant growth regulators by examining sucrose content of immature internodal tissues after treatment application.

Accompanying the increase in sucrose content observed in the varieties CP 65-357, CP 61-37 and NCo 310 was a reduction in endogenous glucose and fructose levels in immature internodal tissues after glyphosate application (Figures 5-10). A significant negative correlation was noted between sucrose and glucose and fructose concentrations in glyphosate treated tissues (Part II). The reduction in endogenous glucose and fructose concentrations

probably indicated a decreased demand for carbohydrate by processes associated with vegetative growth. A decline in the demand for reducing sugars could result in increased amounts of sucrose stored after the application of a growth repressant such as glyphosate.

Sucrose uptake rates did not decline as rapidly in glyphosate treated tissues when compared to control in the experiment (Figures 11-13). However, sucrose uptake rates were significantly higher than control only in NCo 310 (Table 7). Increased sucrose content occurred in those same tissues (Figures 11-13). Although this would seem to be contradictory in terms of sucrose storage, this observation is likely due to a greater proportion of sucrose being stored.

The mode of action of growth repressing plant growth regulators probably reflects a change in source-sink relationships within the sugarcane stalk. By reducing the growth rate of sugarcane (Martin et al., 1976; Dill et al., 1980) these compounds severly diminish the energy requirements of the vegetative apex as a metabolic sink. Subsequently, the need for carbohydrate declined resulting in a decline in glucose and fructose concentrations in immature internodal tissues as observed in this experiment. The reported decline in total soluble acid invertase activity after glyphosine application to sugarcane plants (Alexander, 1976) might then be a decline in that species of the enzyme postulated to function in the regulation of

carbohydrate flow from immature internodal tissues to areas of utilization (Hawker and Hatch, 1965). A net increase in sucrose content of the tissues would be observed since a greater portion of sucrose would be stored in these tissues as was observed in these experiments. The decreased turnover rates of existing sucrose in immature internodal tissues observed after glyphosate application (Hilton et al., 1980) would support this contention.

The soluble fraction of acid invertase (vacuolar and apoplastic) has been reported to decline with increased maturity of the tissues (Hawker and Hatch, 1965). Increased sucrose and reductions in reducing sugar concentrations are also an indication of increased internode maturity (Glasziou and Gayler, 1972). The action of plant growth regulators in causing the above responses in sugarcane probably reflects an accelerated maturity of immature internodal tissue as observed in these experiments. These same effects can be produced under certain environmental conditions. Low temperature and decreased water and nitrogen availability are known to increase sucrose content in sugarcane (Legendre, 1975). The effects of the plant growth regulators tested in these experiments could then be described as accelerating the maturation process of immature internodal tissues by altering source-sink relationships within the sugarcane plant.

A relationship between sucrose percent, CRS/T or

average stalk weight as determined by whole stalk analysis and seedling NR activity was not observed in this experiment (Table 11). Differences among varieties were observed for all variables measured in the experiment (Appendix Table 28). It was postulated that seedlings with high NR activity would also show increased sucrose content at harvest. However, this was not observed in this experiment. Of particular interest was the lack of any correlation in NR activity between seedlings grown in the field and those grown in the greenhouse. This observation was consistent with previous reports (Eck et al., 1975; Deckard and Bush, 1978). This also indicates that the use of in vivo NR activity as a predictor variable for ultimate yield might be questionable due to the unpredictable variation observed under different environmental conditions.

No significant differences were observed in in vivo leaf NR activity between glyphosate treated and untreated CP 65-357, CP 61-37 and NCo310 throughout this experiment. Since no relationship was established between NR activity and sucrose percent and CRS/T, differences in NR activity of plant growth regulator treated and untreated sugarcane might not be expected.

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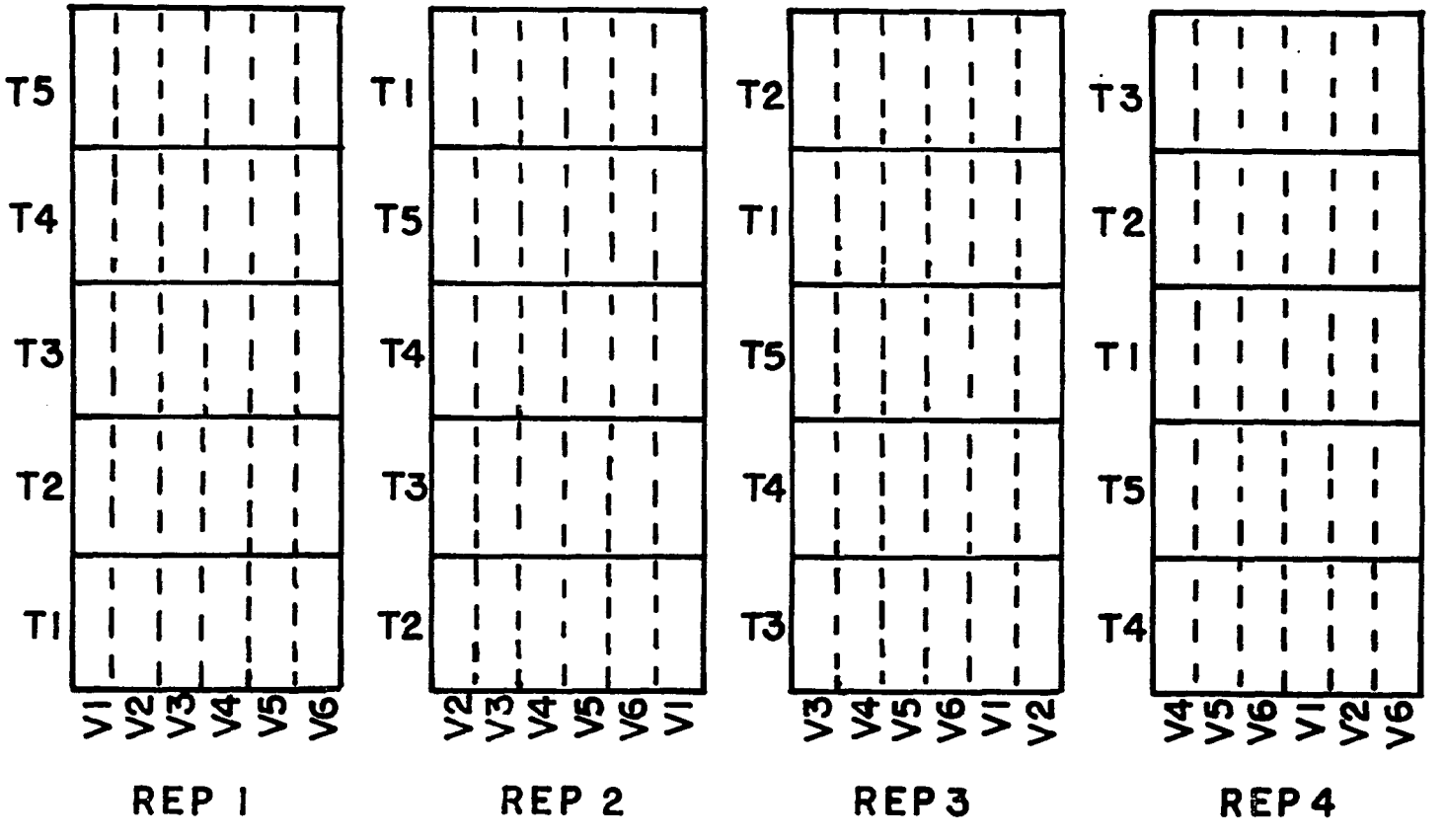
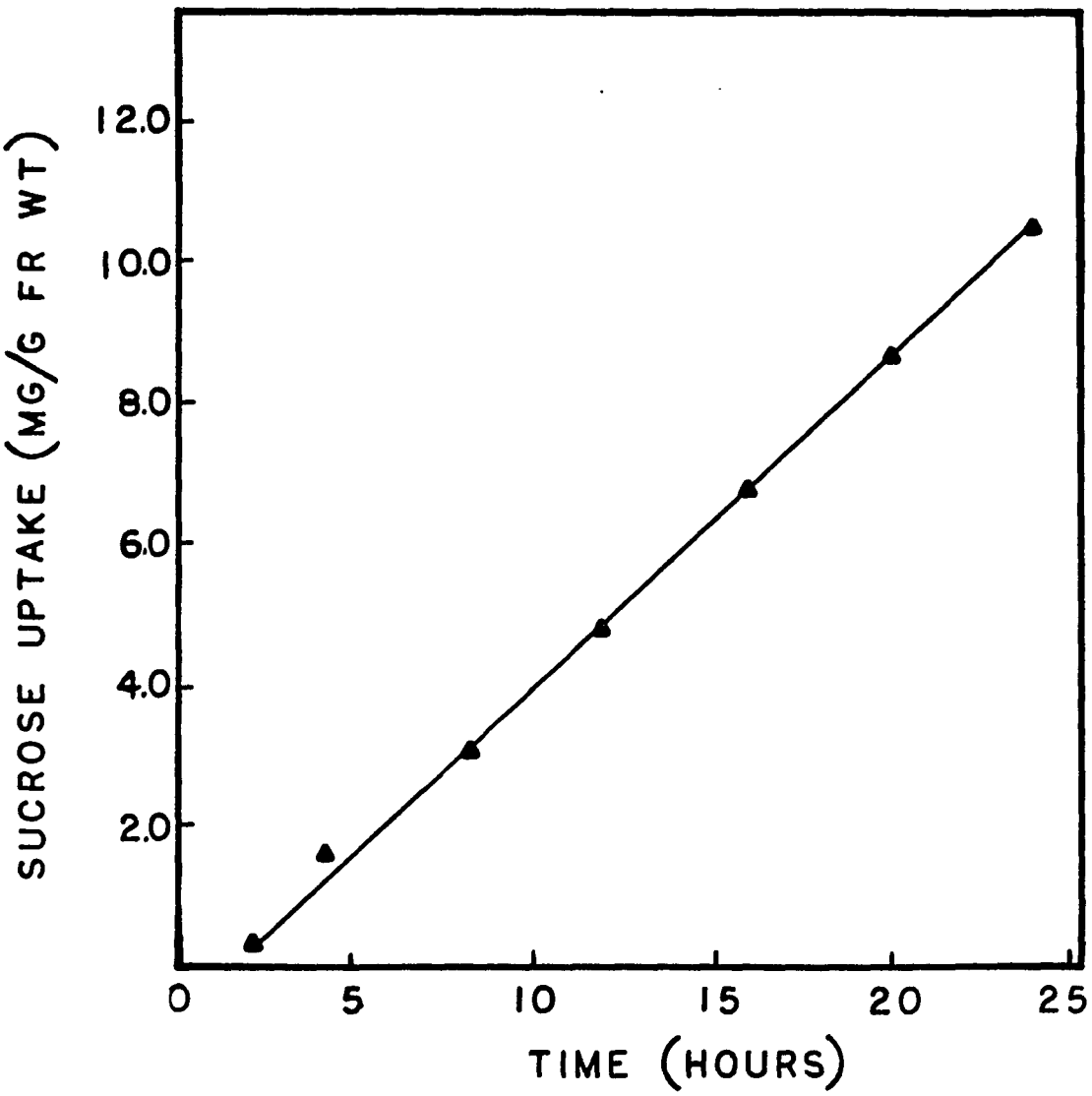
APPENDIX I



Appendix I, Figure 1. Randomization plan for experiments conducted in 1978 and 1979 at the St. Gabriel Experiment Station (V = variety, T = treatment).



Appendix I, Figure 2. Response of sucrose uptake with time. Each point represents the mean of 6 replicates.

Appendix Table 1. Treatments applied to plots at the St. Gabriel Agricultural Experimental Station in the 1978 and 1979 experiments.

Treatment	Product Name	Supplier	Formulation	Rate Used
Control	-	-	-	-
Mefluidide	Embark	3M Corp.	2 lb ai/gallon solution	1.12 kg ai/ha
Ethephon	Ethrel	Union Carbide	2 lb ai/gallon solution	1.12 kg ai/ha
Glyphosine	Polaris	Monsanto Co.	85% soluble powder	3.36 kg ae/ha
Glyphosate	Polado	Monsanto Co.	65% soluble powder	0.45 kg ae/ha

Appendix Table 2. Varieties planted at the St. Gabriel Experiment Station for testing in the 1978 and 1979 experiments.

NCo 310
CP 61-37
L 62-96
CP 65-357

Appendix Table 3. Analysis of variance for the variable sucrose content (mg/g dry weight).

Source	DF	SS	F
Year (Y)	1	3551.9	0.13
Replication (R)	3	32523.4	0.40
Treatment (TR)	4	1506948.5	14.02*
Y x TR	4	535552.6	12.16*
Error A	27	297178.7	
Variety (V)	3	163707.7	3.62*
Y x V	3	170898.9	7.02*
Error B	18	145960.5	
TR x V	12	261784.4	1.96*
Y x TR x V	12	251451.3	2.21*
Error C	72	684210.5	
Time (T)	1	1789517.4	247.87*
Y x T	1	190968.0	26.45*
T^2	1	204629.3	28.34*
Y x T^2	1	39083.9	5.41*
TR x T	4	287005.9	9.38*
Y x TR x T	4	131533.5	4.55*
TR x T^2	4	243715.9	8.44*
Y x TR x T^2	4	165198.3	5.72*
V x T	3	67941.8	3.14*
Y x V x T	3	46194.1	2.13
V x T^2	3	68233.9	3.15*
Y x V x T^2	3	40527.8	1.87
TR x V x T	12	115455.9	1.33
Y x TR x V x T	12	133012.1	1.54
TR x V x T^2	12	184823.1	2.13*
Y x TR x V x T^2	12	135504.5	1.56
CP 65-357, glyphosate vs. control	1	440117.2	52.26*
CP 61-37, glyphosate vs. control	1	278125.3	33.03*
NCo310, glyphosate vs. control	1	396009.0	47.03*
L 62-96, comparison 1	1	275760.2	28.67*
L 62-96, comparison 2	1	96322.9	10.01*
L 62-96, comparison 3	1	36368.3	3.78
L 62-96, comparison 4	1	47731.2	4.96*
Residual Error	640	4620518.9	
Corrected Total	879	20593349.9	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 4. Mg of sucrose per gram dry weight of tissue extracted with ethanol. Data are presented as the mean of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	49.54	49.55	127.48	139.75	378.73	263.17
"	Mefluidide	40.85	78.59	142.72	316.87	434.27	521.87
"	Ethephon	72.34	113.68	229.53	266.59	329.42	398.51
"	Glyphosine	44.04	128.89	313.39	371.07	568.82	469.81
"	Glyphosate	53.26	195.65	292.21	408.70	475.10	469.81
CP 61-37	Control	12.21	11.26	111.42	109.95	363.20	541.50
"	Mefluidide	123.11	79.58	225.69	287.30	335.31	418.56
"	Ethephon	37.91	127.18	277.77	300.69	678.20	286.10
"	Glyphosine	355.01	253.42	369.68	406.01	465.75	458.87
"	Glyphosate	73.38	194.84	385.94	382.32	491.23	292.73
L 62-96	Control	47.87	66.14	76.60	185.84	197.22	188.30
"	Mefluidide	122.60	86.53	174.79	142.54	372.24	563.83
"	Ethephon	34.69	209.35	288.96	290.71	384.30	644.33
"	Glyphosine	39.51	171.06	211.28	305.07	433.97	406.70
"	Glyphosate	21.44	101.33	254.24	306.45	394.92	577.29
CP 65-357	Control	13.29	104.54	165.91	301.82	339.90	204.06
"	Mefluidide	55.29	104.36	248.76	269.31	417.26	517.70
"	Ethephon	98.87	205.11	332.51	236.83	409.12	595.81
"	Glyphosine	38.16	214.34	377.54	422.78	470.31	560.78
"	Glyphosate	46.18	257.95	349.61	466.86	486.66	552.18

Appendix Table 5. Mg of sucrose per gram dry weight of tissue extracted with ethanol. Data are presented as the mean of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	89.95	142.58	238.89	95.46	323.60	235.42
"	Mefluidide	120.70	132.77	175.16	145.22	319.00	260.56
"	Ethephon	119.92	188.47	254.90	287.60	385.14	329.11
"	Glyphosine	161.53	124.25	254.45	189.93	296.94	377.20
"	Glyphosate	90.50	189.41	222.89	265.62	465.28	432.42
CP 61-37	Control	155.90	165.06	288.02	173.69	320.73	325.75
"	Mefluidide	140.42	180.14	220.22	268.45	361.13	418.42
"	Ethephon	166.99	263.25	301.83	290.90	415.82	478.71
"	Glyphosine	218.16	124.09	238.98	322.59	321.13	505.28
"	Glyphosate	114.56	247.89	315.85	333.30	387.25	413.09
L 62-96	Control	141.02	193.90	293.10	157.72	285.02	328.23
"	Mefluidide	131.99	164.86	203.37	178.50	305.56	371.12
"	Ethephon	153.35	208.74	287.23	303.83	400.18	321.71
"	Glyphosine	140.85	146.11	171.53	153.31	306.86	329.82
"	Glyphosate	146.04	237.50	407.36	223.87	472.65	448.42
CP 65-357	Control	119.50	110.92	145.09	231.74	212.88	295.25
"	Mefluidide	153.50	153.84	152.77	119.48	363.29	367.20
"	Ethephon	105.33	180.07	286.40	399.79	306.60	343.27
"	Glyphosine	188.65	200.40	315.30	215.64	345.66	424.98
"	Glyphosate	128.93	152.75	207.23	278.61	394.72	358.54

Appendix Table 6. Analysis of variance for the variable sucrose percent of cane.

Source	DF	SS	F	
Year	1	.0018	7.97	*
Replication	3	.0010	1.76	
Treatment	4	.0019	2.10	
Year x Treatment	4	.0019	2.51	
Error A	27	.0051		
Variety	3	.0036	2.29	
Year x Variety	3	.0036	2.93	
Error B	18	.0074		
Treatment x Variety	12	.0015	0.75	
Year x Treatment x Variety	12	.0016	0.78	
CP 65-357, glyphosate vs. control	1	.0007	3.59	
CP 61-37, glyphosate vs. control	1	.0011	5.64	*
NCo310, glyphosate vs. control	1	.0011	5.64	*
L 62-96, comparison 1	1	.0001	0.51	
L 62-96, comparison 2	1	.0000	0.00	
L 62-96, comparison 3	1	.0002	1.02	
Residual Error	64	.0110		
Corrected Total	151	.0641		

*Significant at $\alpha = 0.05$ level.

Appendix Table 7. Sucrose percent of cane. Data collected from and averaged across 4 replications in 1978.

Variety	Treatment				
	Control	Mefluidide	Ethephon	Glyphosine	Glyphosate
NCo 310	9.16	10.01	10.29	11.71	12.92
CP 61-37	11.94	12.10	12.15	12.27	14.31
L 62-96	13.01	13.37	14.27	13.78	15.08
CP 65-357	14.40	15.82	14.13	15.56	17.02

Appendix Table 8. Sucrose percent of cane. Data collected from and averaged across 4 replications in 1979.

Variety	Treatment				
	Control	Mefluidide	Ethephon	Glyphosine	Glyphosate
NCo 310	12.22	13.69	12.23	12.18	13.26
CP 61-37	14.00	14.62	15.43	13.72	14.87
L 62-96	14.39	16.23	15.26	14.57	14.12
CP 65-357	15.58	14.65	15.03	14.97	15.63

Appendix Table 9. Analysis of variance for the variable CRS/T (kg/ton).

Source	DF	SS	F	
Year	1	1183.6	14.90	*
Replication	3	392.9	1.95	
Treatment	4	662.2	2.08	
Year x Treatment	4	652.9	2.44	
Error A	27	1809.3		
Variety	3	1178.8	2.31	
Year x Variety	3	1146.7	2.83	
Error B	18	2428.0		
Variety x Treatment	12	416.5	0.65	
Year x Variety x Treatment	12	416.83	0.61	
CP 65-357, glyphosate vs. control	1	171.9	3.22	
CP 61-37, glyphosate vs. control	1	382.0	7.18	*
NCo310, glyphosate vs. control	1	446.5	8.38	*
L 62-96, comparison 1	1	26.3	0.49	
L 62-96, comparison 2	1	5.8	0.12	
L 62-96, comparison 3	1	86.7	1.63	
Residual Error	64	3655.6		
Corrected Total	151	21720.3		

*Significant at $\alpha = 0.05$ level.

Appendix Table 10. Average CRS/T (kg per ton) of varieties and treatments averaged across 4 replications in 1978.

Variety	Treatment				
	Control	Mefluidide	Ethephon	Glyphosine	Glyphosate
NCo 310	39.94	45.62	45.52	56.69	66.39
CP 61-37	60.10	60.61	60.93	61.93	75.83
L 62-96	66.86	68.68	74.71	72.50	81.01
CP 65-357	75.85	85.83	75.50	83.82	93.59

Appendix Table 11. Average CRS/T (kg per ton) of varieties and treatments averaged across 4 replications in 1979.

Variety	Treatment				
	Control	Mefluidide	Ethephon	Glyphosine	Glyphosate
NCo 310	63.88	72.34	64.01	63.37	70.54
CP 61-37	76.09	80.54	85.63	73.70	81.84
L 62-96	78.67	91.23	85.54	79.82	76.47
CP 65-357	86.04	80.54	82.66	82.29	86.15

Appendix Table 12. Estimated response equations generated from data collected in the 1978 and 1979 experiments, x = weeks after treatment application.

Treatment	Response Equation
Control	$99.40 + 37.96x - 2.17x^2$
Glyphosate	$97.28 + 87.48x - 4.16x^2$
Glyphosine	$85.32 + 66.92x - 3.49x^2$
Mefluidide	$123.56 + 4.68x + 6.74x^2$
Ethephon	$96.41 + 102.14x - 8.52x^2$

Appendix Table 13. Analysis of variance for the variable glucose content (mg/g dry weight).

Source	DF	SS	F
Year (Y)	1	9620.5	3.13
Replication (R)	3	7514.3	0.81
Treatment (TR)	4	128405.7	10.44*
Y x TR	4	48566.8	7.02*
Error A	27	46684.1	
Variety (V)	3	92715.2	9.79*
Y x V	3	10270.8	1.10
Error B	18	56004.1	
TR x V	12	52999.2	2.97*
Y x TR x V	12	23729.1	1.41
Error C	72	101075.5	
Time (T)	1	116537.3	83.62*
Y x T	1	6932.4	4.97*
T^2	1	687.7	0.49
Y x T^2	1	24144.1	17.33*
TR x T	4	47513.9	8.52*
Y x TR x T	4	42505.6	7.62*
TR x T^2	4	46650.8	8.37*
Y x TR x T^2	4	58825.6	10.55*
V x T	3	15261.2	3.65*
Y x V x T	3	20178.2	4.83*
V x T^2	3	17940.4	4.29*
Y x V x T^2	3	18731.6	4.48*
TR x V x T	12	50440.9	3.02*
Y x TR x V x T	12	31735.3	1.90*
TR x V x T^2	12	65237.1	3.90*
Y x TR x V x T^2	12	31172.8	1.86*
CP 65-357, glyphosate vs. control	1	29403.2	19.36*
CP 61-37, glyphosate vs. control	1	13007.5	8.57*
NCo310, glyphosate vs. control	1	36323.7	23.92*
Residual Error	640	891866.5	
Corrected Total	879	2872132.1	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 14. Mg glucose per gram dry weight of tissue extracted with ethanol. Data are presented as the means of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	132.00	105.65	146.09	145.10	98.79	55.22
"	Mefluidide	165.02	155.58	159.28	113.05	63.01	22.40
"	Ethephon	126.54	183.15	156.31	91.63	35.76	15.30
"	Glyphosine	182.08	121.59	107.23	70.76	35.72	3.68
"	Glyphosate	148.51	63.31	90.92	58.95	45.76	16.93
CP 61-37	Control	142.10	135.12	214.34	173.88	82.04	28.32
"	Mefluidide	128.08	198.23	176.01	120.19	55.50	24.84
"	Ethephon	128.45	197.26	98.89	169.44	10.38	10.40
"	Glyphosine	158.82	104.73	104.49	78.65	58.79	6.83
"	Glyphosate	187.89	166.76	83.47	82.27	33.01	37.60
L 62-96	Control	180.25	162.71	236.12	142.71	112.07	33.82
"	Mefluidide	108.75	177.76	147.99	122.78	75.89	36.90
"	Ethephon	184.77	137.62	104.80	103.86	36.60	9.93
"	Glyphosine	150.26	122.75	139.74	87.88	41.34	14.09
"	Glyphosate	163.28	140.12	76.86	82.98	46.05	12.11
CP 65-357	Control	121.90	113.39	133.80	100.12	48.40	51.81
"	Mefluidide	137.69	132.88	112.21	72.83	45.55	11.82
"	Ethephon	187.34	132.40	113.30	62.63	18.25	14.83
"	Glyphosine	113.26	105.83	77.78	40.82	30.84	9.65
"	Glyphosate	183.71	98.84	59.21	25.34	23.32	6.97

Appendix Table 15. Mg of glucose per gram dry weight of tissue extracted with ethanol. Data are presented as the mean of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	140.95	129.46	154.70	93.04	100.50	97.67
"	Mefluidide	130.44	149.66	142.97	124.50	101.90	122.11
"	Ethephon	169.39	150.79	117.00	91.92	88.30	56.05
"	Glyphosine	143.36	171.18	103.37	117.29	92.44	79.65
"	Glyphosate	126.89	124.61	115.65	85.82	41.79	54.82
CP 61-37	Control	167.65	177.96	138.50	81.75	115.92	60.55
"	Mefluidide	184.31	160.85	137.58	147.92	73.56	65.67
"	Ethephon	147.18	156.21	97.31	58.41	87.06	17.04
"	Glyphosine	163.79	185.51	121.75	63.69	77.92	42.82
"	Glyphosate	198.97	144.36	112.31	78.68	87.16	67.27
L 62-96	Control	140.43	155.65	119.96	150.31	112.57	85.10
"	Mefluidide	160.04	141.85	149.78	93.64	90.89	94.91
"	Ethephon	159.18	134.22	106.91	97.96	78.00	76.28
"	Glyphosine	156.20	145.91	122.72	116.73	117.68	104.22
"	Glyphosate	149.84	128.13	101.09	102.52	65.60	45.57
CP 65-357	Control	153.42	172.12	114.83	82.47	109.48	94.87
"	Mefluidide	151.84	130.64	97.26	92.86	91.27	46.50
"	Ethephon	177.43	131.64	84.56	62.19	56.24	67.20
"	Glyphosine	127.77	129.42	69.39	64.65	68.41	49.64
"	Glyphosate	104.67	94.59	112.69	94.53	40.76	38.01

Appendix Table 16. Analysis of variance for the variable fructose content (mg/g dry weight).

Source	DF	SS	F
Year (Y)	1	5327.5	4.15*
Replication (R)	3	2318.4	1.80
Treatment (TR)	4	74377.4	14.47*
Y x TR	4	9863.3	2.22
Error A	27	29954.4	
Variety (V)	3	29112.5	4.42*
Y x V	3	26005.9	7.78*
Error B	18	20051.4	
TR x V	12	19865.1	1.59
Y x TR x V	12	10557.6	0.82
Error C	72	76905.1	
Time (T)	1	98737.3	94.66*
Y x T	1	6000.8	5.75*
T^2	1	4637.3	4.45*
Y x T^2	1	21066.1	20.20*
TR x T	4	26175.5	8.67*
Y x TR x T	4	9522.6	2.28
TR x T^2	4	32518.1	7.79*
Y x TR x T^2	4	12433.9	2.98*
V x T	3	13905.0	4.44*
Y x V x T	3	7095.2	2.27
V x T^2	3	15622.3	4.99*
Y x V x T^2	3	9408.0	3.01*
TR x V x T	12	27094.5	2.16*
Y x TR x V x T	12	9793.7	0.78
TR x V x T^2	12	26126.7	2.09*
Y x TR x V x T^2	12	8444.9	0.67
CP 65-357, glyphosate vs. control	1	18500.7	17.07*
CP 61-37, glyphosate vs. control	1	12194.6	11.25*
NCo310, glyphosate vs. control	1	23987.4	22.14*
Residual Error	640	667535.5	
Corrected Total	879	1834279.1	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 17. Mg of fructose per gram dry weight of tissue extracted with ethanol. Data are presented as the means of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	130.27	90.17	120.84	126.07	85.72	40.14
"	Mefluidide	127.28	137.78	133.41	92.84	54.24	21.32
"	Ethephon	91.53	177.08	94.70	84.09	45.38	30.22
"	Glyphosine	147.53	101.40	90.18	58.20	37.02	4.34
"	Glyphosate	117.11	58.76	69.40	45.38	43.60	22.14
CP 61-37	Control	142.10	99.12	136.49	114.98	46.74	36.23
"	Mefluidide	93.44	150.56	126.51	93.82	48.72	20.83
"	Ethephon	92.24	149.57	73.11	132.25	10.38	10.40
"	Glyphosine	50.84	183.67	69.02	100.31	36.25	4.44
"	Glyphosate	159.00	117.63	57.54	59.81	24.50	19.01
L 62-96	Control	163.21	139.27	160.12	124.84	88.30	40.65
"	Mefluidide	91.70	138.88	127.66	100.07	63.54	70.88
"	Ethephon	138.14	119.88	89.83	75.65	33.56	12.41
"	Glyphosine	129.21	98.19	127.64	76.16	42.26	12.95
"	Glyphosate	141.86	115.01	71.71	74.21	43.11	13.71
CP 65-357	Control	100.27	97.53	110.95	82.89	44.30	42.71
"	Mefluidide	120.51	106.29	93.27	61.54	23.15	15.74
"	Ethephon	144.68	107.80	97.88	73.78	20.72	23.56
"	Glyphosine	94.70	91.72	68.31	37.59	62.07	15.64
"	Glyphosate	171.62	88.67	40.58	29.72	22.90	6.30

Appendix Table 18. Mg of fructose per gram dry weight of tissue extracted with ethanol. Data are presented as the mean of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	131.34	115.17	123.14	86.29	85.67	97.00
"	Mefluidide	125.94	127.80	124.46	109.92	92.85	116.76
"	Ethephon	140.48	140.94	105.87	76.11	93.09	60.60
"	Glyphosine	128.16	148.90	54.39	102.09	85.28	62.77
"	Glyphosate	117.35	108.40	99.45	71.54	53.04	62.77
CP 61-37	Control	138.05	118.66	93.86	62.86	62.68	49.27
"	Mefluidide	120.00	118.74	97.46	117.76	57.36	53.57
"	Ethephon	116.92	112.31	76.57	46.90	73.57	32.80
"	Glyphosine	78.22	128.50	88.04	44.16	41.51	31.65
"	Glyphosate	138.25	84.52	85.45	25.18	45.24	59.13
L 62-96	Control	116.81	118.08	90.68	116.52	103.48	70.76
"	Mefluidide	125.46	113.63	113.79	79.75	74.00	53.02
"	Ethephon	122.75	107.16	90.12	83.33	63.46	68.76
"	Glyphosine	124.11	114.63	104.00	98.89	88.38	85.90
"	Glyphosate	124.58	97.60	58.75	80.84	54.45	37.98
CP 65-357	Control	122.61	139.16	99.20	66.84	91.36	79.84
"	Mefluidide	125.27	113.24	80.96	84.81	79.61	45.28
"	Ethephon	160.89	115.02	76.19	51.91	63.99	64.29
"	Glyphosine	112.03	114.31	63.94	57.92	64.08	54.53
"	Glyphosate	142.10	88.31	115.48	30.56	48.36	43.33

Appendix Table 19. Analysis of variance for the variable sucrose uptake (mg/g fresh wt per hour).

Source	DF	SS	F
Year (Y)	1	20.411	223*
Replication (R)	3	0.054	0.197
Treatment (TR)	4	0.751	1.182
Y x TR	4	0.095	0.260
Error A	27	2.463	
Variety (V)	3	1.516	16.27*
Y x V	3	0.104	1.14
Error B	18	0.548	
TR x V	12	1.754	2.12
Y x TR x V	12	1.334	1.80
Error C	72	4.446	
Time (T)	1	0.286	5.27*
Y x T	1	1.683	31.03*
T^2	1	0.012	0.22
Y x T^2	1	1.098	20.24*
TR x T	4	1.517	6.99*
Y x TR x T	4	0.593	2.73*
TR x T^2	4	1.134	5.23*
Y x TR x T^2	4	0.351	1.63
V x T	3	0.465	2.86*
Y x V x T	3	0.162	0.99
V x T^2	3	0.292	1.79
Y x V x T^2	3	0.114	0.70
TR x V x T	12	0.792	1.22
Y x TR x V x T	12	1.282	1.97*
TR x V x T^2	12	0.776	1.19
Y x TR x V x T^2	12	1.120	1.72
CP 65-357, glyphosate vs. control	1	0.037	0.61
CP 61-37, glyphosate vs. control	1	0.011	0.18
NCo310, glyphosate vs. control	1	0.321	5.25*
Residual Error	640	38.41	
Corrected Total	879	84.278	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 20. Sucrose uptake rates[1/] in internodal tissue. Data are presented as the means of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	0.913	0.591	0.326	0.428	0.363	0.571
"	Mefluidide	1.665	0.995	0.382	0.416	0.569	0.322
"	Ethephon	0.709	0.920	0.799	0.483	0.240	0.363
"	Glyphosine	0.331	0.503	0.542	0.479	0.356	0.461
"	Glyphosate	0.657	1.158	0.567	0.498	0.676	0.557
CP 61-37	Control	0.888	0.955	0.360	0.458	0.409	0.451
"	Mefluidide	0.405	0.850	0.265	0.373	0.416	0.337
"	Ethephon	0.781	1.058	0.924	0.569	0.681	0.554
"	Glyphosine	0.210	0.857	0.456	0.582	0.780	0.532
"	Glyphosate	0.753	0.712	0.402	0.606	0.639	0.304
L 62-96	Control	0.934	1.008	0.271	0.433	0.335	0.229
"	Mefluidide	0.294	0.741	0.464	0.425	0.366	0.559
"	Ethephon	0.526	0.893	0.440	0.549	0.410	0.468
"	Glyphosine	0.142	0.603	0.543	0.442	0.265	0.332
"	Glyphosate	0.763	0.599	0.373	0.522	0.594	0.790
CP 65-356	Control	0.895	0.896	0.623	0.542	0.551	0.410
"	Mefluidide	0.644	0.753	0.772	0.425	0.584	0.394
"	Ethephon	0.698	0.620	0.593	0.534	0.376	0.554
"	Glyphosine	0.948	0.785	0.705	0.519	0.742	0.793
"	Glyphosate	0.858	0.824	0.498	0.627	0.600	0.640

[1/] Data are expressed as mg sucrose accumulated per g fr wt per hr.

Appendix Table 21. Sucrose uptake rates[1/] in internodal tissue. Data are represented as the means of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	0.360	0.310	0.346	0.168	0.001	0.228
"	Mefluidide	0.203	0.394	0.253	0.177	0.211	0.178
"	Ethephon	0.223	0.356	0.340	0.372	0.187	0.153
"	Glyphosine	0.154	0.359	0.178	0.329	0.039	0.175
"	Glyphosate	0.291	0.302	0.263	0.353	0.256	0.314
CP 61-37	Control	0.312	0.286	0.391	0.245	0.319	0.349
"	Mefluidide	0.125	0.185	0.310	0.397	0.266	0.174
"	Ethephon	0.246	0.171	0.538	0.392	0.246	0.302
"	Glyphosine	0.250	0.287	0.277	0.245	0.186	0.281
"	Glyphosate	0.229	0.445	0.436	0.122	0.332	0.300
L 62-96	Control	0.203	0.246	0.462	0.234	0.255	0.139
"	Mefluidide	0.023	0.354	0.323	0.209	0.153	0.191
"	Ethephon	0.243	0.262	0.215	0.237	0.340	0.203
"	Glyphosine	0.049	0.258	0.265	0.115	0.203	0.113
"	Glyphosate	0.234	0.399	0.300	0.358	0.205	0.119
CP 65-357	Control	0.409	0.386	0.286	0.186	0.312	0.360
"	Mefluidide	0.364	0.377	0.402	0.243	0.349	0.029
"	Ethephon	0.074	0.377	0.543	0.458	0.363	0.125
"	Glyphosine	0.289	0.283	0.355	0.482	0.383	0.277
"	Glyphosate	0.377	0.123	0.421	0.337	0.336	0.284

[1/]Data are expressed as mg sucrose accumulated per g fr wt per hr.

Appendix Table 22. Analysis of variance for the variable sucrose content (mg/g fresh weight).

Source	DF	SS	F
Year (Y)	1	2762.1	4.17*
Replication (R)	3	1101.4	0.55
Treatment (TR)	4	41760.1	15.76*
Y x TR	4	9647.9	5.98*
Error A	27	10882.4	
Variety (V)	3	16746.8	6.62*
Y x V	3	7544.1	4.45*
Error B	18	10163.8	
TR x V	12	6116.9	1.24
Y x TR x V	12	3331.8	0.64
Error C	72	31269.0	
Time (T)	1	27369.0	105.10*
Y x T	1	1207.8	4.63*
T^2	1	27.8	0.11
Y x T^2	1	186.8	0.72
TR x T	4	7408.7	7.11*
Y x TR x T	4	5219.3	5.01*
TR x T^2	4	5427.5	5.21*
Y x TR x T^2	4	7018.5	6.74*
V x T	3	855.4	1.09
Y x V x T	3	953.2	1.20
V x T^2	3	1030.2	1.32
Y x V x T^2	3	1343.0	1.72
TR x V x T	12	6285.4	2.01*
Y x TR x V x T	12	8106.0	2.59*
TR x V x T^2	12	10217.4	3.27*
Y x TR x V x T^2	12	9106.2	2.91*
CP 65-357, glyphosate vs. control	1	15415.1	49.93*
CP 61-37, glyphosate vs. control	1	7666.6	24.83*
NCo310, glyphosate vs. control	1	11677.5	37.82*
Residual Error	640	166715.2	
Corrected Total	879	784644.4	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 23. Mg of sucrose per gram fresh weight of tissue extracted with ethanol. Data are presented as the means of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	3.40	4.23	8.13	10.09	42.96	37.92
"	Mefluidide	2.31	4.68	10.03	31.69	61.88	81.88
"	Ethephon	4.58	13.87	25.46	25.18	41.51	60.75
"	Glyphosine	2.25	9.84	32.00	45.10	96.49	76.15
"	Glyphosate	2.50	17.28	25.13	57.03	70.12	81.00
CP 61-37	Control	0.94	0.93	7.94	8.65	45.73	61.68
"	Mefluidide	3.66	5.44	19.63	35.27	36.72	73.40
"	Ethephon	2.75	11.43	22.76	30.44	104.81	31.00
"	Glyphosine	2.69	31.10	36.62	49.27	61.13	62.47
"	Glyphosate	9.17	15.87	37.37	44.78	71.46	66.20
L 62-96	Control	1.78	4.14	5.60	16.95	18.01	22.32
"	Mefluidide	1.21	5.25	11.94	12.71	51.40	79.67
"	Ethephon	1.81	15.21	25.85	32.55	63.43	133.39
"	Glyphosine	2.03	10.56	22.40	27.01	60.49	64.13
"	Glyphosate	1.00	10.68	23.61	34.89	63.45	106.97
CP 65-357	Control	0.63	11.44	15.03	32.49	52.40	31.46
"	Mefluidide	2.22	8.22	29.72	33.16	86.29	114.90
"	Ethephon	7.25	17.24	36.22	25.39	67.53	96.10
"	Glyphosine	2.16	16.53	40.41	55.43	101.58	107.44
"	Glyphosate	2.37	23.22	39.34	61.59	114.89	86.31

Appendix Table 24. Mg of sucrose per gram fresh weight of tissue extracted with ethanol. Data are presented as the mean of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	6.11	10.80	17.23	8.79	32.53	26.25
"	Mefluidide	8.79	10.17	14.11	9.46	29.89	25.76
"	Ethephon	8.31	11.92	25.43	30.37	42.07	39.51
"	Glyphosine	11.74	9.04	23.50	13.04	29.78	47.66
"	Glyphosate	6.04	14.39	21.98	30.16	60.77	62.92
CP 61-37	Control	12.03	13.59	34.11	14.08	40.58	49.16
"	Mefluidide	10.28	16.62	26.12	32.32	46.62	58.65
"	Ethephon	11.69	24.89	38.45	44.96	58.04	80.10
"	Glyphosine	19.56	9.86	25.88	31.32	40.93	92.38
"	Glyphosate	8.75	27.42	33.62	56.19	47.87	65.56
L 62-96	Control	11.03	17.05	32.07	13.97	32.84	40.22
"	Mefluidide	10.21	13.98	19.17	19.73	33.25	46.27
"	Ethephon	11.50	20.10	30.54	31.73	51.87	46.01
"	Glyphosine	10.70	12.92	15.00	17.08	31.25	40.48
"	Glyphosate	10.47	18.73	55.88	28.13	78.53	78.27
CP 65-357	Control	10.23	8.83	16.02	33.32	29.68	42.79
"	Mefluidide	14.53	14.66	19.39	14.58	41.34	73.36
"	Ethephon	6.85	17.42	40.44	48.71	43.43	54.13
"	Glyphosine	18.75	24.58	45.92	30.55	64.91	66.11
"	Glyphosate	8.96	16.53	24.81	42.45	68.56	68.33

Appendix Table 25. Analysis of variance for the variable nitrate reductase activity (μmoles NO_2 produced per g fresh wt per hour).

Source	DF	SS	F
Year (Y)	1	0.929	84.5*
Replication (R)	3	0.473	14.34*
Treatment (TR)	4	0.227	5.16*
Y x TR	4	0.023	0.73
Error A	27	0.296	
Variety (V)	3	0.109	0.96
Y x V	3	0.529	12.11*
Error B	18	0.262	
TR x V	12	0.126	0.35
Y x TR x V	12	0.168	0.85
Error C	72	0.782	
Time (T)	1	0.072	5.51*
Y x T	1	0.082	6.29*
T^2	1	0.051	3.92*
Y x T^2	1	0.007	0.57
TR x T	4	0.172	3.30*
Y x TR x T	4	0.067	1.30
TR x T^2	4	0.128	2.46*
Y x TR x T^2	4	0.059	1.14
V x T	3	0.042	1.08
Y x V x T	3	0.148	3.80*
V x T^2	3	0.074	1.89
Y x V x T^2	3	0.085	2.18*
TR x V x T	12	0.052	0.34
Y x TR x V x T	12	0.268	1.72
TR x V x T^2	12	0.045	0.29
Y x TR x V x T^2	12	0.273	1.74
CP 65-357, glyphosate vs. control	1	0.008	0.60
CP 61-37, glyphosate vs. control	1	0.001	0.07
NCo310, glyphosate vs. control	1	0.048	3.59
Residual Error	715	9.310	
Corrected Total	954	15.350	

*Significant at the $\alpha = 0.05$ level.

Appendix Table 26. *In vivo* NR activity.[1/] Data are presented as the means of 4 replications, year 1978.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	0.244	0.116	0.205	0.202	0.215	0.235
"	Mefluidide	0.237	0.099	0.317	0.201	0.266	0.413
"	Ethephon	0.136	0.141	0.321	0.238	0.234	0.321
"	Glyphosine	0.248	0.109	0.252	0.251	0.498	0.262
"	Glyphosate	0.167	0.163	0.251	0.234	0.269	0.194
CP 61-37	Control	0.322	0.187	0.214	0.134	0.238	0.185
"	Mefluidide	0.269	0.211	0.301	0.217	0.188	0.203
"	Ethephon	0.258	0.106	0.272	0.140	0.115	0.267
"	Glyphosine	0.228	0.224	0.263	0.304	0.201	0.240
"	Glyphosate	0.208	0.214	0.248	0.182	0.202	0.196
L 62-96	Control	0.286	0.169	0.235	0.230	0.142	0.266
"	Mefluidide	0.200	0.153	0.221	0.311	0.279	0.295
"	Ethephon	0.204	0.195	0.365	0.258	0.287	0.425
"	Glyphosine	0.333	0.191	0.269	0.196	0.568	0.298
"	Glyphosate	0.319	0.194	0.329	0.238	0.322	0.337
CP 65-357	Control	0.378	0.180	0.244	0.208	0.154	0.289
"	Mefluidide	0.211	0.116	0.284	0.230	0.192	0.156
"	Ethephon	0.270	0.192	0.536	0.314	0.065	0.358
"	Glyphosine	0.195	0.225	0.315	0.309	0.228	0.354
"	Glyphosate	0.289	0.175	0.417	0.187	0.259	0.390

[1/] Data are expressed as μmoles NO_2^- produced per g fr wt per hr.

Appendix Table 27. *In vivo* NR activity.[1/] Data are represented as the means of 4 replications, year 1979.

Variety	Treatment	Weeks After Application					
		0	1	2	3	5	7
NCo 310	Control	0.322	0.223	0.203	0.161	0.088	0.129
"	Mefluidide	0.093	0.293	0.139	0.132	0.126	0.082
"	Ethephon	0.232	0.320	0.199	0.176	0.106	0.093
"	Glyphosine	0.325	0.181	0.158	0.404	0.140	0.117
"	Glyphosate	0.286	0.300	0.235	0.125	0.154	0.061
CP 61-37	Control	0.338	0.184	0.150	0.187	0.181	0.180
"	Mefluidide	0.237	0.234	0.126	0.265	0.132	0.220
"	Ethephon	0.109	0.272	0.202	0.254	0.235	0.144
"	Glyphosine	0.342	0.216	0.197	0.278	0.156	0.150
"	Glyphosate	0.247	0.176	0.144	0.306	0.161	0.097
L 62-96	Control	0.243	0.159	0.069	0.083	0.083	0.100
"	Mefluidide	0.208	0.159	0.078	0.084	0.105	0.149
"	Ethephon	0.230	0.249	0.055	0.102	0.109	0.142
"	Glyphosine	0.224	0.122	0.065	0.128	0.125	0.155
"	Glyphosate	0.179	0.168	0.051	0.136	0.136	0.067
CP 65-357	Control	0.154	0.216	0.103	0.273	0.144	0.155
"	Mefluidide	0.390	0.146	0.097	0.190	0.192	0.122
"	Ethephon	0.232	0.247	0.161	0.269	0.211	0.233
"	Glyphosine	0.206	0.297	0.110	0.301	0.198	0.258
"	Glyphosate	0.188	0.258	0.160	0.287	0.189	0.143

[1/] Data are expressed as μmoles NO_2^- produced per g fr wt per hr.

Appendix Table 28 . Analysis of variance for the variables included in the study of *in vivo* leaf NR activity and sugar yield.

Variable	Variety DF	Variety SS	Error DF	Error SS	F
NR Activity [1/] (Field Seedlings)	17	0.55	54	0.62	2.80 *
NR Activity [1/] (Greenhouse Seedlings)	17	4.56	54	3.71	3.83 *
Sucrose Percent (%)	17	434.60	54	23.10	52.85 *
CRS/T (kg/ton)	17	74752.00	54	46.99	50.52 *
Average Stalk Weight (kg)	17	29.33	54	1.28	72.65 *

[1/] μmoles NO_2^- produced per gram fresh weight per hour.

* Significant at the = 0.05 level.

APPENDIX II

Using Area Estimation for Treatment Comparisons in Regression-Type Models

Analyzing treatment differences in the analysis of covariance with intra-class regression models has been accomplished via the examination of slope heterogeneity (Patel, 1976). Using models for the one-way and two-way analyses, the author used type IV sums of squares in the GLM procedure of SAS (Statistical Analysis Systems - Barr et al, 1976) in which an overall test of slope heterogeneity using generated cell means was made. Significance of this test was taken as an indication of overall slope heterogeneity, while a lack of significance indicated invariance of treatments with respect to the covariable. Patel indicated that if indeed the a priori test of slope heterogeneity was nonsignificant that comparisons of the main effects and interaction in the model would then be appropriate. In the instance where overall slope heterogeneity was detected, the author again created cell means for appropriate comparisons of cell slopes in the model.

An alternative method of examining treatment differences in such models as described above will be discussed here.

In the case of covariance analysis with intra-class regression effects one can view the treatment relationships to the concomitant variable as follows:

$$\text{Model, } Y_{ij} = \mu + \alpha_i + \gamma X_{ij} + \beta_i X_{ij} + \epsilon_{ij}$$

where i= 1, 2 and j 1, 2, 3 and 4.

Where $\alpha_1+\beta_1$ and $\alpha_2+\beta_2$ represent the equations of the line for two levels of an effect α as Y is regressed on X (Figure 1). In this instance, examining the main effects might not be the appropriate test of treatment differences, especially if the treatments are not invariant with respect to the concomitant variable. A test of slope heterogeneity would be one alternative, however, one could actually compare the areas under the two lines and formulate a test of treatment differences. By integrating (across the values of the concomitant variable) the respective equations presented in Figure 1 and setting them equal to one another one can accomplish this.

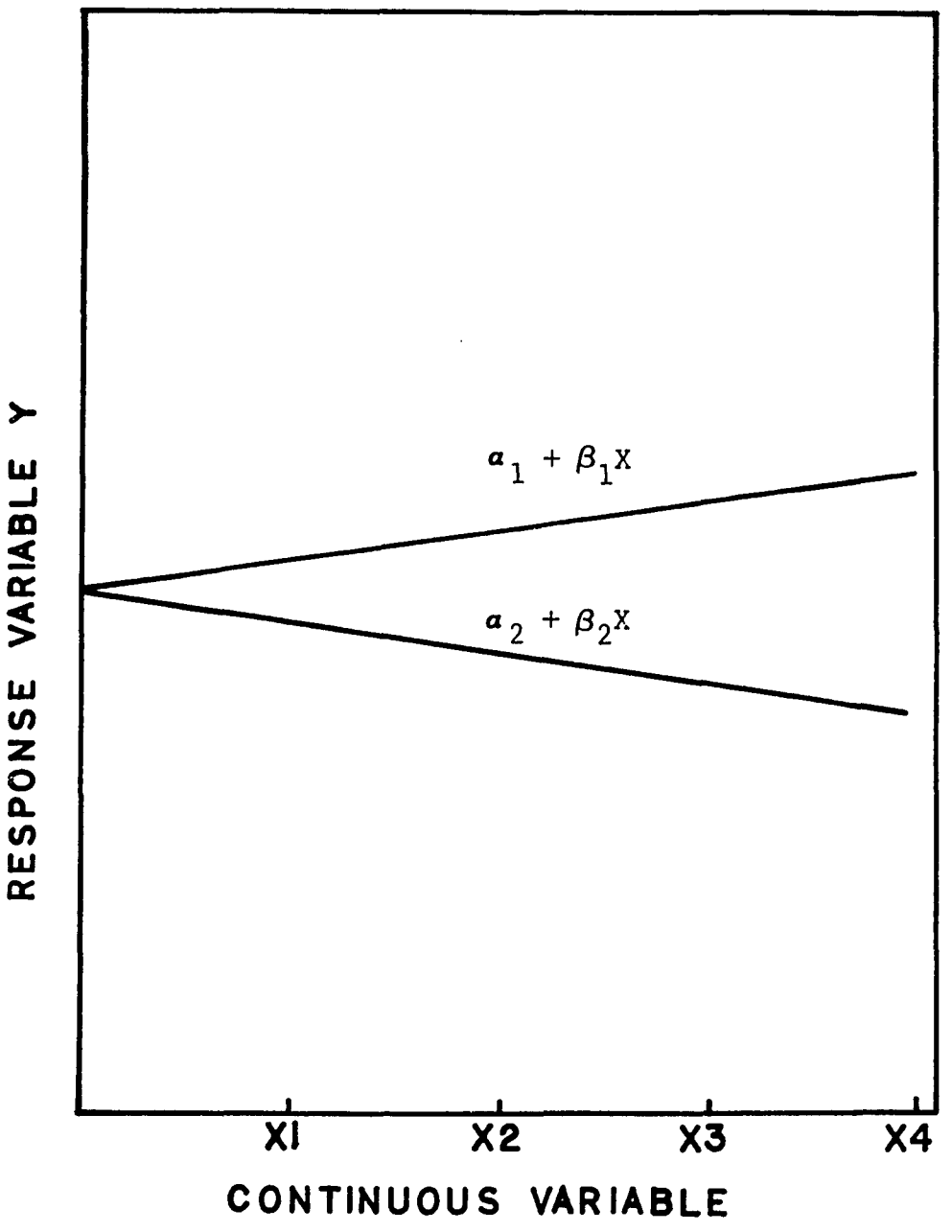
$$\int_{X_4}^{X_1} (\alpha_1+\beta_1 X)\ f(x)dx = \int_{X_4}^{X_1} (\alpha_2+\beta_2 X)\ f(x)dx$$

Where f(x) represents the probability distribution function (p.d.f.) of the concomitant variable, X. It must be noted here that the p.d.f. of X can be omitted from the above integration only in those cases where it is uniformly distributed across the range of X in question. The integration is carried out below assuming X is uniformly distributed in this instance.

(1) $$\int_{X_4}^{X_1} (\alpha_1+\beta_1 X)\ f(x)dx = \int_{X_4}^{X_1} (\alpha_2+\beta_2 X)\ f(x)dx$$

(2) $$\left(\alpha_1 X\Big|_{X_1}^{X_4} + \tfrac{1}{2}\beta_1 X^2\Big|_{X_1}^{X_4}\right) = \left(\alpha_2 X\Big|_{X_1}^{X_4} + \tfrac{1}{2}\beta_2 X^2\Big|_{X_1}^{X_4}\right)$$

(3) $$(X_4-X_1)\alpha_1 + \tfrac{1}{2}(X_4^2-X_1^2)\beta_1 = (X_4-X_1)\alpha_2 + \tfrac{1}{2}(X_4^2-X_1^2)\beta_2.$$



Appendix II, Figure 1. Linear regression of Y on X.

By grouping the above equations to the left of the equal sign and setting the result equal to zero we now obtain:

$$(X_4-X_1)\,\alpha_1 + \tfrac{1}{2}(X_4^2-X_1^2)\beta_1 - (X_4-X_1)\alpha_2 - \tfrac{1}{2}(X_4^2-X_1^2)\beta_2 = 0.$$

In establishing a hypothesis matrix (vector in this instance), (X_4-X_1) is the coefficient associated with α_1 and α_2, while $1/2\ (X_4^2-X_1^2)$ becomes the coefficient associated with β_1 and β_2 with the sign of the coefficients designated in the previous equation.

The hypothesis being tested is that the difference in area under the lines of interest is zero across a specified range of the concomitant variable. By using CONTRAST statements in SAS, a vector or matrix of coefficients is established. This vector (or matrix) is the L vector GLM uses to generate sums of squares for specific tests of hypothesis. SAS calculates the sum of squares as follows:

$$SS_h = (LB)'\ (L(X'X)^{-}L')^{-1}(LB).$$

Where L is a lxp vector of coefficients generated through the previous integration and B is the pxl estimated parameters vector. Note that LB is the estimate for the difference in area under the lines of interest, and the test of hypothesis is, therefore,

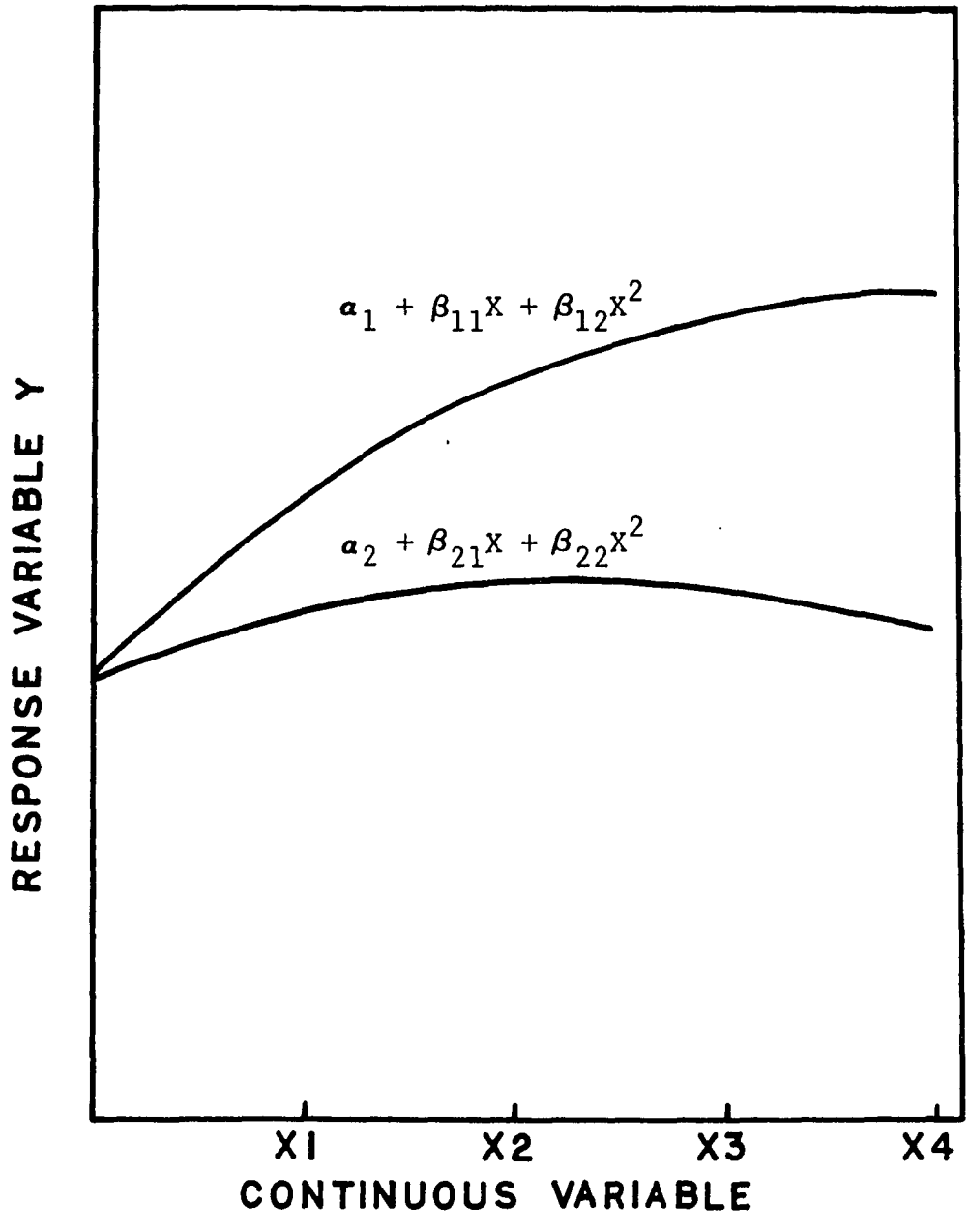
$$H_o: LB = 0.$$

This technique can also be applied to nonlinear models. In such instances, expansion of the model with the appropriate

expansion of the response equations are utilized. An example of the quadratic case is shown in Figure 2. Area estimations would be as follows:

$$\int_{X_1}^{X_4}(\alpha_1 + \beta_{11}X + \beta_{12}X^2)\ f(x)dx = \int_{X_1}^{X_4}(\alpha_1 + \beta_{21}X + \beta_{22}X^2)\ f(x)dx.$$

It should also be noted that tests of hypothesis pertaining to specific ranges of the covariate in the experiment can also be constructed for the nonlinear case.



Appendix II, Figure 2. Non-linear regression of Y on X.

APPENDIX III

GLOSSARY

Brix. The percent by weight of soluble solids in a solution at a specified temperature.

Commercially Recoverable Sugar per Ton (CRS/T). An estimate of the amount of sucrose extractable fron one ton of harvested sugarcane. Calculated from sucrose percent and observed brix in extracted juice utilizing varietal and commercial correction factors.

In Vivo Nitrate Reductase Activity (NR). The amount of nitrite produced by plant tissue segments incubated in a buffered nitrate solution. NR activity is expressed in moles NO_2^- produced per g fr wt per hr and calculated as follows (volume corrected):

$$NR = \frac{\mu\text{moles } NO_2^- \text{ (80 min)} - \mu\text{moles } NO_2^- \text{ (20 min)}}{\text{tissue weight}}$$

Reducing Sugar. Any sugar that because of a free or potentially free aldehyde or ketone groups, possess the property of readily reducing alkaline solutions of many metallic salts such as iron, copper or silver.

Sucrose Enhancement. An increase in the percent sucrose in the stalk of sugarcane.

Sucrose Percent. The percent by weight of sucrose in juice extracted in whole stalk sugar analysis.

Sucrose Uptake. The uptake of sucrose from a solution by slices of plant tissue. Sucrose uptake is expressed in mg per g fr wt per hour and calculated as follows (volume corrected):

$$\text{Sucrose Uptake} = \frac{\text{Sucrose (mg) 12hr} - \text{Sucrose (mg) 4hr}}{\text{(8hr) (tissue weight)}}$$

VITA

Gerald Matthew Dill, Jr. was born in Houma, Louisiana on December 12, 1952. His family moved to Crowley, Louisiana in August, 1955 where he attended parochial schools and graduated from Notre Dame High School in May, 1970. The author entered Louisiana State University in September, 1970 and recieved a B. S. degree from that institution in December, 1974.

In June, 1975 Gerald enrolled as a graduate student at Louisiana State University in the Department of Plant Pathology and recieved a M. S. degree in May, 1977. The author continued his graduate studies in the Department of Plant Pathology and Crop Physiology at Louisiana State University. The author also enrolled as a graduate student in the Department of Experimental Statistics and recieved a Masters of Applied Statistics degree in December, 1980.

The author is married to Jan C. Massa of Houma, Louisiana. They now have three children, Byron, Stuart and Elizabeth.

EXAMINATION AND THESIS REPORT

Candidate: Gerald Matthew Dill, Jr.

Major Field: Plant Pathology (Crop Physiology)

Title of Thesis: The Influence of Exogenous Plant Growth Regulators on Sucrose Accumulation in Sugarcane (Saccharum sp.)

Approved:

Freddie A Martin
Major Professor and Chairman

James D. Traynham
Dean of the Graduate School

EXAMINING COMMITTEE:

Marc Allen Cohn

William J. Blackmon

Kenneth L Koonce

John B. Baker

Thomas R. Hargrove

Date of Examination:

May 13, 1981